

Where now on arms control?

Last week's collapse of the intermediate-range missile talks at Geneva emphasizes the need to keep talking about strategic weapons and to devise a broader framework next month in Stockholm.

THE Soviet Union's walk-out last week from the arms control negotiations at Geneva may not be quite the calamity that it seems: that is the bright side of the coin. For this development, well flagged in advance as the probable response to the first appearance in Europe of the cruise and Pershing II missiles which are, in turn, the response of the North Atlantic Treaty Organization (NATO) to the deployment of SS20 missiles in the 1970s, may not be final. And even if it is, it has been plain for months that the negotiations at Geneva were getting nowhere, especially on questions such as whether British and French nuclear weapons should be counted with others capable of being used against European targets. And anyway, the argument goes, it is surely sensible that the artificial distinction between strategic weapons based on the numerical value of their carriers' range should be abandoned. For has it not been plain for the past two years that the negotiations on missiles of intermediate range should be thrown together with those (also at Geneva) on strategic weapons called START (for Strategic Arms Reduction Talks)? See *Nature* 297, 444; 1982 for an example of this much repeated argument.

The other side of the coin is that there is no reason why any of these good things should happen. Now that the Soviet Union has walked away from the negotiations on intermediate-range weapons (although START continues), and has begun building extra missile bases in Eastern Europe outside its own territory, the momentum of arms deployment in Europe has been further augmented. Even if, as seems likely from contradictory signals in the past few weeks, the Soviet Union's leadership is divided, with the military people pushing for counterpoises to the new NATO missiles and the civilians urging caution, the urge to complete the new deployment will be strong. And while last week's statement by Mr Yuri Andropov, suggesting that the Soviet side would not return to the same negotiations at Geneva, left the door open for the continuation of the same negotiations by some other means, it seems certain that the Soviet Union will not now take the initiative in designing a better framework for further talks. Why should it, when it has more to gain by waiting for the slow deployment over the next three years of the new cruise and Pershing II missiles, and for the public "peace" demonstrations likely to embarrass NATO governments throughout that period?

Blame

Who, in the circumstances, is to blame? This muck-raking question is not profitless but could be a guide to what lies ahead. The simple answer is that both parties to the Geneva negotiations, the Soviet Union and the United States, are inexcusably at fault. The Soviet Union has stolidly but cynically insisted that its deployment of SS20s capable only of reaching European targets in the West (China is also covered) was simply a normal component of military preparedness. It was inevitable that NATO should have threatened in December 1979 that its own nuclear arsenals would be reinforced if some agreement on the SS20s could not be reached. The government of the Soviet Union can have been in no doubt that its deployment of its missiles, if left unmatched, would have challenged the cornerstone of NATO, the guarantee by the United States of the security of Western Europe.

The faults of the United States are different in kind but equally distressing. First, President Jimmy Carter shrank from presenting the SALT II agreement to the US Congress on the grounds of

displeasure about Soviet military support for an unpopular government in Afghanistan. Second, the new Reagan Administration (which took office on 20 January 1981) behaved during its first year as if negotiations on arms control (for which it had no stomach) could be postponed indefinitely — forgetting both its solemn promises (for example, under the Non-Proliferation Treaty) and the political needs of allied governments in Western Europe. And when negotiations inevitably began, eighteen months later than they might have done, the United States restricted its own freedom of manoeuvre by declaring publicly a succession of ill-considered goals. The "zero option" for the intermediate range negotiations (no cruise and no Pershing II missiles, all SS20s taken away), everybody's ultimate ideal, was destined from the start to be as unattainable as events have shown but also to raise the awkward question of the British and French nuclear forces. During the past two years, this proposal and its successors, by being made public, have made the United States negotiating position needlessly rigid. Mr Eugene Rostow, the director of the US Arms Control and Disarmament Agency unceremoniously fired a year ago, may have been lucky. Who would wish to be an international negotiator when the negotiations that really matter are those between the elements of the US Government that have eventually materialized as "new" negotiating positions?

Misreading

The fear that the US Government (towards the end of the Carter presidency as more recently) may have misread the function and underestimated the importance of US relations with the Soviet Union is now almost as serious a source of alarm elsewhere as the accumulation of nuclear weapons in and around Europe. The days of *détente* (the mid-1970s) are a long way behind us, and were always unreal. The rot began in 1977, when Mr Jimmy Carter swept into office with the laudable ambition of blessing all peoples everywhere with a modicum of government for the people by the people — even if that meant ramming it down their throats. Although more realistic, even hard-bitten, the Reagan Administration has never been able to arrive at a workable policy on relations with the Soviet Union. The administration says publicly and repeatedly that the Soviet system is an abomination and, by means of embargoes on equipment that might be used for building pipelines, does its best to ensure that it fails to earn a living for itself. This is legitimate enough (which is not to say wise) but needs to be offset by more than mere declarations to the contrary when awkward jibes are made about its seriousness on arms control negotiations.

Meanwhile, nothing much will happen on arms control until next month, when there will convene in Stockholm a meeting of the signatories of the Helsinki accords, the loose association of European and allied states which agreed at Madrid earlier this year that there should be a further attempt to find ways of reducing the risks of war in Europe. By then, the participants at Geneva may be in a more sober mood, willing to see their business carried on within a different framework. There are two obvious choices. One, likely to be most popular with the participants at Stockholm, is to embark on the negotiation of an arms control instrument so different from that whose negotiation has come to nothing at Geneva that neither side will be held to have lost face by joining in. The notion that there might be a nuclear-free zone in



Central Europe, now more than two decades old, has resurfaced from several directions in recent months and would in principle make it possible to attain President Reagan's ideal zero option by other than bilateral negotiations. The snag (which does not attend all proposals for such zones) is that the effect of such a proposal would put the West at a serious disadvantage unless there were a more general and a substantial reduction of nuclear weapons everywhere — an objective still a long way off.

This is why the best outcome of the Stockholm meeting would be an agreement on two points — that there should be negotiations on nuclear weapons in general embracing both the START and intermediate-range missile talks and that all five declared nuclear powers, not just the biggest, should somehow take part. The case for dealing with other than strategic weapons is amply illustrated by the questions that have arisen during the past two years at Geneva. Are British and French nuclear weapons strategic, tactical or something else? Are aircraft capable of carrying nuclear weapons to be counted as if they were missiles? What about the devices euphemistically called battlefield weapons?

But would not the involvement of extra parties with such diverse interests merely complicate negotiations that are already difficult enough? That has been the conventional wisdom so far, and indeed there is every reason why the Soviet Union and the United States should be made to push ahead on START in the hope of getting some kind of agreement by 1985, when SALT II lapses automatically (and when the signatories of the Non-Proliferation Treaty will be asking what the nuclear powers have done about arms control). But it is also plain, from the disappointments at Geneva, that the variety of the means by which nuclear weapons can be delivered is now so great that the hope of negotiating separately on different systems — of agreeing on the trade-off between SS20 and Pershing II as if aircraft or artillery did not exist — is nugatory. Indeed, there is a good chance that the sheer diversity of interest of the three minor nuclear powers would be a help and not a hindrance, if only because the negotiations would then be less black-and-white. And they have one thing in common with the larger powers — all of them would prefer that nuclear weapons should be markedly reduced in number. □

Bombs are not new

ABC's horror film on nuclear war, should be shown until nobody is surprised.

IN the long run, the success of arms control negotiations will be determined by what ordinary people think about the urgency of the cause, and by the way in which they convey to governments their opinions on what should be done. The reception last week in the United States of ABC television's film "The Day After" is, in this connection, depressing. The event was preceded by solemn warnings that children of a tender age (or indeed, any age) should not be allowed to watch the film without the presence of adults who might be able to comfort and explain. The United States Government also thought it proper that Mr George Shultz, the Secretary of State, should appear in public afterwards to promise that he and his colleagues are as surely against nuclear war as anybody else. The experience seems momentarily to have been cathartic for a large proportion of 250 million people — a proof that nuclear warfare would be both devastating and horrible.

But who ever thought that it could be otherwise? Since a decade after Hiroshima and Nagasaki, the nature of what would happen has been clear. This was the period when committees as different as those sponsored by the Government of India and the United Nations set about the grisly task of extrapolating from what was known of the effects of isolated 20 kiloton explosions to estimate the effects of all-out attack with megaton weapons. The same documents circulated in the United States, and were as widely read. Have they been more easily forgotten, obliterated perhaps by more recent traumas? If so, ABC should show its film again, and then again, for that is what arms control is about. □

Wind blows cool

Windmills, like wave generators before them, seem to be falling out of favour in Britain.

IS the British Government's wind power programme in the doldrums? Earlier this year, the Department of Energy's advisory committee on research and development put wind at the top of the list of renewable sources of energy, saying at the same time that wave power is unlikely to be economic. As a consequence, the department's support for wind power rose by a half to £4.8 million (1983–84), while wave power fell by two thirds to £1 million. But according to Sir Sam Edwards, newly appointed part-time scientific adviser to Mr Peter Walker, also a new boy as Secretary of State for Energy, one of Sir Sam's first jobs will be to look at the rising cost of big wind turbines such as the department's £5 million, 3-MW station now under construction in the Orkneys, off the coast of Scotland. Nothing has been decided, but there is every chance that wind power will go the way of wave power fairly soon. On paper, windmills look good, but British opinion has cooled as the big American devices have been found to be plagued by problems such as cracked shafts and blades.

There seems also to have been some reassessment among British wind enthusiasts of forecasts of electricity production costs made a few years ago. The most obvious difficulty is that while small machines producing less than 250 kW peak power have been shown to function reliably, it is agreed that it will be necessary to build machines like the Orkney 60-m diameter windmill and thus stretch technology to the limit if competitive electricity is to be generated. Sites for windmills are another difficulty, and utility officials are painfully aware that it would be necessary to find 1,000 sites merely to match the output of a single pressurized water reactor. Even with the fuss generated by nuclear proposals, the prospect of 1,000 contentious public inquiries about windmills is daunting.

What, in these circumstances, should the Department of Energy decide? The British nationalized utility, the Central Electricity Generating Board, is building a 4-MW windmill in Kent that will produce electricity at a cost three or four times that of electricity generated in bulk. Replicating that experiment is unlikely to be profitable, for there can be no assurance that the benefits of mass production are anywhere within reach. There is more to be learned from the accumulation of experience with smaller machines in circumstances where they can be used economically for supplying electricity to isolated communities, where their use in Britain is at present inhibited by the antiquated rule that levies local taxes at the rate of 6 per cent of capital cost a year. A modest subsidy, as in the United States, would be more appropriate. Will Mr Walker fight for that? His reputation within the government of which he is a member is that of a liberal; windmills would be an endearing and not too dangerous a cause.

Whether the British Government, with or without help from its new scientific adviser, will be able to chart a future for the industry on which the United Kingdom will have to rely for extra electricity generation in the decades ahead is another matter. The decision that the British Government would sanction the building of one nuclear power station a year for the decade now half gone was first announced soon after the general election of 1979. It is true that the demand for electricity has remained obdurately flat since then under the twin influences of economic recession and improved efficiency. But the proposal to build a single pressurized water reactor is still enmeshed in the public inquiry being held in Suffolk. The inquiry will survive robustly into the new year and Mr Walker, if he is lucky, may have moved on to another post before the time comes for the occupant of his post to respond. The difficulty then, of course, will be that of receiving the opinion by the inspector at the inquiry courteously when it is bound to seem that the inquiry has usurped the authority over energy planning that the Department of Energy exists to exercise. To have a few windmills somewhere (though not at £4 million each) will provide some light relief. □

Spacelab project

West German involvement faces uncertain future

THE launch earlier this week from Cape Canaveral in Florida of the shuttle craft carrying the Spacelab experiment probably marks a turning point in collaboration on space projects between the European Space Agency (ESA) and the National Aeronautics and Space Administration (NASA) in the United States.

European pleasure that the instrument has finally been carried aloft is real enough, if tinged with regret at the delay there has been and the less than satisfactory state of the communications system originally planned for the transmission of data in real time between Spacelab and the ground.

But there is also some resentment that the first version of Spacelab has already become the property of NASA and that further use of it by European experimenters will have to be paid for. This is why Dr Heinz Riesenhuber, the West German minister for research and technology, has taken to describing Spacelab as one of the most generous of all gifts to the United States. West Germany, which has been the chief contributor to the DM1,000 million (£250 million) cost of Spacelab and largely responsible for its development and construction is especially concerned.

The apparently faultless construction of Spacelab will have enhanced even the West German reputation for precision engineering, thus authenticating ESA as a fit partner for collaboration on space ventures. But there are questions, especially in France, about whether the cost of the machine might more profitably have been spent indigenously.

At the beginning of the venture in 1972, the circumstances were very different. Then, the potential of the Ariane rocket system seemed less than immediate, while the prospectus then advertised for the space shuttle offered the prospect that the first version of Spacelab would stay aloft for much longer than the five days now planned. One senior French scientist said last month that he "felt sorry" about West Germany's investment in Spacelab.

Part of the trouble is that the costs of the project have escalated. While part of the philosophy of the design has been to use as many standard components as possible, such components have not always been inexpensive. One official in Bonn explained last month how gas cylinders costing DM600 each had turned out to cost more than DM100,000 each after they had been fitted out with the accompanying sheaf of documentation required for certification.

Among the European members of ESA, West German interest in the design and development of orbital machines is matched only by French enthusiasm for the

Ariane launching system.

According to spokesmen at the Bundesministerium für Forschung und Technologie (BMFT — the ministry for research and technology) in Bonn, the objectives of West German policy in space hitherto have been to acquire and demonstrate a competence in the construction of satellites and other space vehicles, to develop the means for using these commercially and to prepare for the time when ESA might have an independent competence in space.

West German achievements in the field

Max-Planck-Gesellschaft

Closer ties with universities

THE Max-Planck-Gesellschaft (MPG), West Germany's largest academic research organization outside the universities, will have a new president when Heinz Staab takes over from Reimar Lüst in July 1984. The MPG has an annual budget of DM950 million (£240 million), of which 6 per cent comes from internal funds and 47 per cent each as lump sums from the Federal Ministry of Research and Development and from the regions (Länder).

Reimar Lüst, scientific director of the European Space Research Organization from 1962 to 1964, returns to become general president of its successor, the European Space Agency, for four years from July 1983. Here Lüst faces a challenging transition period involving decisions on, for example, the future of scientific satellites, applications, particularly in telecommunications, and the development of the launcher. A major question concerns participation in a space station.

Lüst has led MPG adroitly through a period of adjustment to the twilight of the West German economic miracle. Since his appointment in 1972, MPG funds have just kept pace with inflation.

Adaptation has been achieved both by execution and creation. While maintaining its traditional emphasis on the promotion of basic research, of excellence and of the work of gifted individuals, MPG has closed 12 departments but opened 10 more in its 55 institutes. New institutes include one for quantum optics at Munich, an offshoot of the plasma physics institute, and one for polymer research in the federal republic's chemical heartland at Mainz on the Rhine. The institute at Cologne has also been given strong support in the development of plant genetic engineering, providing a focus of excellence unique in Europe.

Heinz Staab's own institute for medical

are considerable. The point has already been reached at which, after the launch of the next direct broadcasting satellite in 1985, responsibility for further communications satellites will pass to the Ministry of Posts in Bonn. West Germany is chiefly responsible for the substantial X-ray satellite called Rosat due to be launched in 1987, as well as being principal contributor to the Galileo mission to Jupiter due in 1985.

Even so, policy in the years ahead is likely now to be closely scrutinized in Bonn. While the new largely Christian Democrat coalition does not carry its belief that industry should meet a larger share of the cost of research and development to the point of declining to support long-term projects, Dr Werner Gries said last month in Bonn that the government would be issuing a new policy statement "before the end of the year".

John Maddox

research in Heidelberg, which will shortly have lost three of its five directors, may well also find a new role possibly related more directly to the complex of surrounding biomedical centres at Heidelberg.

MPG is in the process of introducing a system of extramural supervisory boards (Fachbeiräte) with strong international membership to make reviewing procedures more stringent. There is also a programme of 20 five-year trial directorships which gives younger scientists the chance to show their potential for leadership. MPG's other contribution to solving the problems of young scientists, is the Otto Hahn prizes for overseas work.

Heinz Staab, who is 57 years old and who originally qualified in both medicine and chemistry, heads the department for organic chemistry at the Heidelberg institute. MPG has agreed that he should continue to spend a quarter of his time on his own "bio-organic-chemical" research into intermolecular relations of significance in biological processes. A particular emphasis is, for example, on hydrogen-bonding in model systems.

Staab has a reputation for fast thinking and brilliant public speaking in German and English. He has been a member of the Deutsche Forschungsgemeinschaft, of the Alexander von Humboldt Foundation and of the committee of the German national academic advisory body, the Wissenschaftsrat. Since 1966, he has been a member of the Minerva Society which promotes scientific links with Israel and since 1977 a governor of the Weizmann Institute. He is the elected president of the German Chemical Society.

Staab sees little possibility for change in the present financial situation. He plans to continue present policies, but also to strengthen MPG's links with the universities.

Sarah Tooze

Gene therapy

Quick fixes not on the cards

Washington

GENE therapy in humans, in recent months a popular topic for moral pronouncements from theologians, philosophers and activists, continues to face substantial technical obstacles as well. And even when the techniques become available, only a few of the 3,000 known genetic disorders are likely to be treatable and some of the most familiar and most debilitating disorders such as thalassaemia and sickle-cell anaemia are unlikely to be among the early successes.

"Exaggerated expectations as well as exaggerated fears are very common", said Dr James Wyngaarden, director of the National Institutes of Health (NIH), which last week sponsored a public forum on human gene therapy. Only 300 genetic diseases have been defined biochemically, and fewer than a score of human genes of potential importance in treating genetic defects have been cloned. But more important, according to Dr French Anderson, director of the Laboratory of Molecular Hematology at NIH, there are still no methods for delivering genes to human cells, ensuring their expression and ensuring the safety of such procedures.

Anderson and other researchers who spoke at the forum predicted that the first applications in humans will be attempts to correct "classic" inborn errors of metabolism — such as phenylketonuria and Lesch-Nyhan syndrome — that involve a single missing enzyme, leading to the accumulation of a toxic substance in the body. These disorders would be particularly amenable to gene therapy because neither the regulation nor the location in the body of the introduced gene would matter; so long as the missing gene can be inserted and made to function sufficiently well, it makes no difference where in the body it is functioning. The problem of targeting to a specific organ is also avoided if the disorder can be corrected in a relatively accessible organ, such as bone marrow, which can be removed, treated and then replaced.

Diseases such as diabetes, thalassaemia and coagulation disorders, which require precise regulation of the amount of enzyme product produced, will be far less tractable. The limitations that classic transformation techniques place on the number of transformed cells that can reasonably be introduced further narrows the field. According to Dr Joseph Schulman of George Washington University Medical Center, this means that the first successes will most probably come in treating disorders where the body exerts a natural selective pressure in favour of the transformed cells. Adenosine deaminase (ADA) deficiency and Lesch-Nyhan syndrome are promising candidates. Both are also of intense concern because of their debili-

tating effects and the dismal lack of conventional treatments. ADA deficiency causes a serious immune disorder; Lesch-Nyhan, which is a deficiency in the enzyme hypoxanthine-guanine phosphoribosyl transferase, results in an accumulation of uric acid with a disastrous toll on the kidneys and bizarre self-destructive behaviour that often requires the patient to be physically restrained.

Experience so far with animal models of gene therapy, notably the preimplantation alteration of mouse embryos by micro-injection of genes, may have only a limited application in humans. Apart from the high rate of failure in the mouse experiments and the ethical considerations of germ-line alterations that such techniques would raise in human use, Schulman noted that ultimately the questions of safety and effectiveness can only be answered by research in humans.

On the question of germ-line alteration — the target earlier this year of an apocalyptically worded petition instigated by anti-genetic-engineering activist Jeremy Rifkin and signed by a broad spectrum of US clergymen — both researchers and

ethicists who spoke last week urged caution and the need for careful thought about the implications. John Fletcher, assistant to the director of NIH for bioethics and an Episcopal minister, said that although he was satisfied with the "system of ethics and morality in place to handle somatic cell alteration, a similar system had not been developed for germ-line alterations. But", he added, "I am in favour of keeping an open mind."

Several researchers emphasized a dimension of the moral debate that has been less obvious in recent discussions — the viewpoint of the victims of genetic disease and the propriety of society eschewing possible solutions to their condition. "For all the talk about scientists' pushing to do gene therapy", Anderson said, "the one thing that isn't discussed is the pressure from the patients themselves." And Ola Huntley, whose personal experience with sickle-cell anaemia in her three children has led her to a professional involvement in counselling sickle-cell patients, expressed her side of the moral conflict simply and directly: "I am angry that anyone presumes to deny my children the essential genetic treatment of a genetic disease. I see such persons as simplistic moralists."

Stephen Budiansky

Medical ethics

INSERM sets up forum

PRESIDENT François Mitterrand may open a hornets' nest on Friday, when he presides at the first session of the new ethics committee of the French medical research council INSERM. Mercifully, the session will be short and probably formal, but when the committee is in full flight next year the stings may come thick and fast.

The ethics committee has been given a very broad brief, enabling it to study "moral problems raised by research in biology, medicine and health" where these concern "the individual, social groups or the whole society". The committee may also concern itself with anything that troubles the public mind in relation to biological research, a formula that leaves open the question of the use of animals.

In true democratic spirit, Mitterrand has ensured that all possible conflicting groups are represented on the committee, which is divided into three sections: in the first section, five representatives of the "principal philosophical families", Catholicism, Protestantism, Judaism, Mohammedanism and Communism (an interesting twist) nominated by Mitterrand; in the second section, fifteen chosen for their competence and interest in ethical issues, nominated by various bodies such as the National Assembly, the Senate and the Court of Appeal; and in the third, fifteen scientists nominated by research institutions. From the last two sections, a working party of eight will be nominated. The president of the whole committee is to

be Professor Jean Bernard, president of the Academy of Sciences.

Already, this structure has led to argument: why should Mitterrand name our experts, a senior Catholic asked recently? What would trade unions say if Mitterrand was free to name their representatives on negotiating committees? Why should communism be represented on equal terms with religion? And why were none of the first section of the committee — essentially, the experts in ethics — allowed to be members of the working group, which will no doubt be the heart of the committee? This smacks of technocracy, and hints that the committee will be "soft" on the scientists, says Jesuit Paul Valadier, editor of the magazine *Etudes*. Certainly, whatever its recommendations, President Mitterrand's presence on Friday will give them political weight.

No-one is in much doubt that the first issues to be touched by the committee will concern *in vitro* fertilization and the use of fetal tissue; there is more doubt, however, over what conclusions the committee will reach. Some therefore feel that its role will be much more important in stimulating debate and organizing public discussion than in reaching judgements. This, indeed, was one of the goals of INSERM director-general Philippe Lazar in establishing the committee in the first place. But ultimately it will be up to the committee itself to decide exactly how openly it will operate.

Robert Walgate

Science in Vietnam

Soviet Union extends agreement

SOVIET scientific institutions will "participate more broadly" in Vietnamese research projects under an agreement now signed. The collaborative programme will entail close coordination of the state plans of the two countries in seven areas — agriculture, energy, metallurgy, engineering, chemicals and petrochemicals, transport and communications and geological surveying. Special emphasis is placed on the "production potential" of Vietnam and the rational use of labour and natural resources, especially energy and water.

The agreement was signed with full diplomatic ritual on 31 October as the high-point of the visit to Vietnam of Politburo member Gaider Aliev, considered by many as a possible successor to Mr Yuri Andropov. To a considerable extent, it is an updating of a similar agreement signed in November 1979.

Among other things, the treaty continues the arrangement under which the University of Kishenev, which specializes in training students from developing countries, will continue to take Vietnamese. The first Vietnamese DSc at Kishenev was awarded this year for a thesis on fast-growing soya beans devised to allow for three harvests a year in South-East Asia.

Similar training schemes for scientific "cadres" will be expanded under the new agreement, as well as vocational training schemes in Soviet factories and construction sites. Criticisms of these schemes by Western commentators during the past two years as a means of recruiting unskilled labour have been vigorously denied by the Soviet Union, which last year produced a special public relations film, which was subsequently screened by Vietnamese Television, intended to combat these reports.

Under the new agreement, the Soviet side will also help to establish and equip research laboratories in Vietnamese institutes and colleges. Work will continue on a "modern material and technical base" for research in Vietnam, and the transfer of technical documents and research data from the Soviet Union to Vietnam will be ensured. Soviet experts will in particular participate in the geological survey of the country and its offshore waters, in feasibility studies for the establishment of steel and tin industries and for the industrial use of the Da River, and in establishing a pharmaceutical industry.

A major problem for the Soviet side — which is referred to only obliquely in the agreement — is that Vietnamese science and technology, even on its own assessment, is extremely disorganized. Last August, the party newspaper *Nhan Dan* noted that party resolutions on science and technology were frequently not "concretized". The management and organization of research needed streamlining. Scientific information was not being disseminated widely enough and the criteria and regulations relating to quality control were frequently ignored.

Vietnam is, of course, still recovering from a prolonged war and, over the past eight years, much Soviet aid has gone on getting the economy working again. Only last August, for example, Soviet experts were able to complete repairs to the Dalat nuclear reactor, constructed with the help of the United States in the former South Vietnam.

Not all shortcomings, however, are consequences of the war. There have been several unexplained delays in establishing the outward forms of Socialist science. A State Prize Commission was established

only in September 1982, and the Vietnamese Union of Scientific and Technical Associations held its founding congress only in March this year.

An even more surprising gap — considering the extensive aid that the Soviet Union has extended to Vietnam — has been the neglect of the Russian language. Even Hanoi Polytechnic, which over the past few years has been extensively reconstructed and developed with Soviet aid, acquired a Russian language department only at the beginning of last month.

Vera Rich

White House science

Keyworth adds new blood

Washington

DR George Keyworth, President Reagan's science adviser, last week announced changes among senior personnel of the White House Office of Science and Technology Policy (OSTP). A series of departures has enabled Dr Keyworth to appoint a new deputy director of the office and five new assistant directors. A sixth assistant director will be named later.

OSTP's new deputy director is to be John McTague, a physical chemist and chairman of the National Synchrotron Light Source Department at the Brookhaven National Laboratory. Other outsiders brought into OSTP are Richard Johnson, a member of the Lockheed Palo Alto Research Laboratory, and Maurice Roesch, an active colonel in the Marine Corps. Johnson will become assistant director for space science and technology and Roesch assistant director for defence technology and systems.

Ralph DeVries, already an acting assistant director, is confirmed as assistant director for general science, and two senior policy analysts at OSTP, Wallace Kornack and James Ling, are promoted to assistant director. Kornack will be in charge of energy, natural resources and international affairs; Ling will cover institutional relations and — until a sixth assistant director is named — life sciences.

The new appointments mark a significant change in the structure of OSTP which, until recently, had only three assistant directors. Under the new arrangements, three areas — institutional relations, the life sciences and space science and technology — have been recognized for the first time as separate areas meriting their own assistant directors.

In recent months, OSTP has come under strong criticism by the life sciences community for its apparent emphasis on the physical sciences. The creation of an assistant directorship for institutional relations reflects OSTP's growing interest in reforming the national laboratories and strengthening their collaboration with industry and the universities. Peter David

Sea-cow relics for museum

BERING Island in the Komodorskie group of islands in the Bering Sea, the only recorded habitat of Steller's sea-cow (*Rhytina stelleri*), is to have its own exhibit of the bones of this now-extinct mammal. A virtually complete skeleton, discovered in the summer of 1983 by a seventh-grade school-boy "Sasha" (Aleksandr) Grekov, has been authenticated by the Palaeontological Institute of the Soviet Academy of Sciences and will be assembled and housed in the Komodorskie Branch of the Kamchatka provincial museum.

So far, only six complete skeletons of *Rhytina* have been on public display, in Moscow, Leningrad, New York and other locations remote from the original habitat. The sea-cow was originally named "kapustnik" (cabbage-eater) by Vitus Bering's naturalist Georg Wilhelm Steller, because it browsed the prolific sea-kale of

the islands. The animal was discovered in 1759 and is thought to have become extinct by 1786, due to the depredations of the *promyshleyniki* — the Russian hunters who found the Komodorskie group a convenient source of free meat for their expeditions.

According to *Pravda*, the director of the Kamchatka Museum is enthusiastic about the new exhibit. This is somewhat ironic, because if the Kamchatka local authorities had been more cooperative in the mid-eighteenth century, *R. stelleri* might never have become extinct. In 1755, a Russian mining engineer, Retr Yakovlev, who had been prospecting for copper in the Komodorskie group, appealed to the Kamchatka authorities to take steps to halt the extermination of the "kapustnik". The appeal, however, fell on deaf ears.

Vera Rich

US research tax credits

Congress welcomes new law

Washington

FAR-reaching legislation designed to encourage American high-technology industries and to provide better research facilities at universities will be high on the agenda when Congress reconvenes in January. After lengthy talks with the Treasury and with private industry, Senator John Danforth (Republican, Missouri) has introduced a bill offering new tax advantages to businesses that increase their investment in research and development and donate scientific equipment to universities.

One of the main objectives of the bill, the High Technology Research and Scientific Education Act, would be to extend the system of tax credits for research and development introduced in 1981 and due to expire in December 1985. The existing system, which has proved immensely popular with industry, offers tax incentives to businesses able to demonstrate a significant increase in their research spending. The new bill would make the credits a

permanent part of the tax code and would allow newly-formed companies to qualify.

The bill also proposes using tax benefits to encourage industries to sponsor basic research by universities. Corporations supporting basic research in universities would be able to write off against tax some 25 per cent of the value of any payments they make to universities above a basic "maintenance-of-effort" floor. The floor would be calculated as either the average payment to universities by the corporation over a three-year period or one per cent of its average research budget.

Finally, the act would extend the scope of the tax credits already available to companies contributing scientific equipment to universities. Equipment used for education rather than research would be eligible for the first time, as would computer software. And companies donating repair and maintenance contracts along with the donations would be able to include those costs in their tax credit claims.

Peter David

Hazardous chemicals

US agency loses further friend

Washington

The Reagan Administration is taking action to rebut charges that it has neglected occupational safety. The Occupational Safety and Health Administration (OSHA) last week announced wide-ranging new regulations that will require companies to give employees more information about hazardous chemicals used in the workplace. Thorne Auchter, OSHA's director, claims that the regulation will cost manufacturers some \$600 million and represents the most significant regulatory action in the agency's history.

The regulations, which will cover 14 million employees in some 300,000 companies, will be phased in over three years. By November 1985, manufacturers and importers of chemicals will have to carry out a formal assessment of the hazards of the chemicals they sell. Their products will have to be clearly labelled with the chemicals' names and appropriate hazard warnings; and data sheets will have to be supplied with details of the chemicals and appropriate safety measures.

By 1986, all manufacturing industries will be required to have labelled chemical containers and developed extensive training and educational procedures for their employees. Companies will have to produce written plans for their training and communication efforts.

Promulgation of the new regulations comes at a time when the Reagan Administration and OSHA in particular have come under mounting criticism. Auchter pointed out that the regulation came only

three weeks after the publication of a temporary emergency standard reducing the previous permissible level of exposure to asbestos fibres by 75 per cent.

But criticism of OSHA's record on occupational safety is likely to continue. The announcement of the hazardous chemical regulations was immediately followed by a lawsuit in which AFL-CIO (the body that represents US labour unions) and individual unions representing steel, chemical and automobile workers challenged what they described as a "giant loophole".

AFL-CIO complains that the new regulation embodies provisions under which companies could refuse to divulge the nature of a chemical in order to protect a trade secret, although the information could be demanded in a health emergency.

The regulation's critics also point out that the new rule does not cover service industries and that it preempts existing state laws which are often stricter.

OSHA has already had a difficult time under the Reagan Administration. At congressional hearings last September, Auchter came under strong attack for having delayed action to regulate ethylene dibromide, a widely used fumigant and a potent animal carcinogen. In testimony, Auchter explained his failure to act by claiming that OSHA could not have made an emergency regulation stick in court — a claim contradicted by OSHA documents unearthed by congressional investigators (see *Nature* 22 September, p.263).

Peter David

UK research councils

Making do with less

PROFESSOR John Kingman, chairman of the UK Science and Engineering Research Council (SERC), said last week that important scientific opportunities are being lost in order to save relatively small sums of public money. Launching the council's annual report for 1982-83, Professor Kingman seemed chiefly concerned with how independent investigators could keep their projects alive when funds are short and costs increasing.

Although it is not yet known what proportion of next year's science budget will be allocated to the council, the Advisory Board for the Research Councils last year recommended a gradual transfer of funds from other research councils to SERC. But more immediate problems, chiefly the cost of paying subscriptions to international organizations, mean that SERC is far from optimistic.

One way, Kingman says, to improve the provision for strategic research would be to persuade more industrial companies to become involved in projects such as the Alvey programme in information technology. This programme, supported by the Department of Trade and Industry and industrial companies, has now started definition studies for four large-scale demonstration projects, and others will soon be commissioned.

In other areas, such as ground-based astronomy and synchrotron radiation, the council hopes to develop more links with European partners. But Professor Kingman warned that Britain is in danger of getting out of step with potential collaborators: while most European countries are increasing their support for basic science, Britain is increasingly looking for immediate results.

Kingman defended the role of the SERC as a provider for research in universities and polytechnics rather than as a source of ideas for British industry. It was, he said, essential for the future of science in Britain that long-term building projects should not be curtailed.

The contraction in the universities has not apparently reduced the number or the quality of research grant applications to SERC. But the proportion of grant applications that the council can support is decreasing, and its Science Board will soon be rejecting 30 per cent of the top-grade proposals that would normally be assured of support. The board supports training and research in core areas of British science: biology, chemistry, mathematics and physics.

During the past year the board has changed the way it allocated research grants. It is now taking an active role in developing research themes that it wants to support, rather than responding passively

to requests from scientists for support. More central planning of university-based research also seems to be implied by the decision to establish a special directorate to orchestrate the contribution that universities can make in information technology.

Financial restraints meant that last year SERC supported fewer studentships and advanced course studentships. The number offered in 1982 was 3,178, compared with 3,600 in 1980. Another significant policy change has been agreed in the way SERC will decide what postgraduate studentships to support. Responsibility is to be devolved to the council's separate

subject boards; already the Engineering Board has decided to increase the level of advanced course training that it provides.

In future years there may be an even more difficult partitioning of the science budget to be made, as restructuring costs and capital expenditure within the Agricultural and Food Research Council and the Natural Environmental Research Council press against the aspirations of SERC. Professor Kingman said last week that any diversion of funds away from SERC to meet costs within other councils would amount to "fining the efficient".

Tim Beardsley

Huge drug damages

Washington

A FEDERAL jury in Georgia has ordered Eli Lilly & Co. to pay \$6 million in punitive damages for the death of a woman who took the anti-inflammatory drug Oraflex (benoxaprofen). The drug was ordered to be taken off the market in Britain, where it was sold under the name Opren, in August 1982 after reports of 61 deaths among users of the drug (see *Nature* 298, 597; 1982). On the same day, Lilly suspended sales worldwide.

The damages were the largest dollar amount ever awarded in a drug liability case. Lilly officials said they would file an immediate appeal.

Even before the drug was taken off the market, it had become the centre of a controversy about Lilly's heavy promotion of the product and its failure to report to the US Food and Drug Administration — while US approval was pending last spring — about deaths among users elsewhere in the world.

The Georgia verdict was the first of more than 100 suits pending against Lilly in connection with Oraflex. Many of those cases involve adverse reactions other than death. One earlier case in West Germany was found in Lilly's favour last month.

Stephen Budiansky

Israeli development

To stand alone or deal?

Rehovot

ISRAEL'S science-based industry was responsible for the export of more than \$1,000 million worth of locally-developed products last year, but as this sector grows ever more important, so does the question of its future development. Specifically, policy-makers are trying to decide whether Israel should try to go it alone in the expansion of its science-based industry, or whether emphasis should be put on persuading high-tech companies to set up subsidiaries in this country.

Scitex, with headquarters on the outskirts of Tel Aviv, is one example of what can be accomplished by a purely Israeli high-tech company. After a brief stint on military-related projects, Scitex turned to the development and manufacture of interactive systems for use in graphic arts, mapping and computer-aided design and manufacture.

In the absence of a domestic market for its "gadgets", which sell at \$500,000 and up, Scitex must sell overseas, and it manages to do so very successfully despite competition from West Germany and Britain. *Time* magazine, for example, uses Scitex equipment to computerize the makeup of its pages.

Executive vice president Arthur Low said the company has learned to exploit the skills of Israel's scientists and technologists, who contribute more to the country by working for Scitex, he adds, than they would by "providing the missing piece in a puzzle" whose parameters are defined overseas. Moreover, once such a foreign company had made what use it could of Israeli talent, "it might well move on if it found more attractive conditions elsewhere". Scitex serves instead as the home base for subsidiary companies in the United States, Western Europe and Japan. At any time, one-third of its Israeli personnel are working overseas, gaining valuable experience of potential markets in the process.

Another important Israeli-based enterprise is Ormat Turbines Ltd in Yavne, south of Tel Aviv. Its turbines, which can run on almost any source of heat, are used

to power installations in inaccessible mountain tops and deserts. Ormat has used its technology to produce electricity from geothermal wells in the United States and solar ponds around the Dead Sea.

Encouraged by his own experience, Ormat's founder, Mr Yehuda Bronicki, argues that Israeli companies are capable of turning out sophisticated products that can compete with those of high-tech enterprises anywhere in the world. But relative isolation from major markets means that they must make extra efforts to keep in touch with the needs of their customers. This is why Ormat, like Scitex, has established subsidiaries abroad and regularly sends its research staff on overseas tours of duty.

Like high-tech companies elsewhere, Israeli enterprises devote a significant percentage of their income to research and development — in 1982, 15 per cent of \$15 million at Ormat and 15 per cent of \$50 million at Scitex. In addition, such companies receive money from the office of Professor Aryeh Lavie, Chief Scientist of the Ministry of Industry and Commerce.

While Israeli-based high-tech companies are flourishing, so too are local branches of overseas companies. Thus Intel, which eleven years ago opened a semiconductor design centre in Haifa, has now embarked upon a \$130 million programme to build a wafer fabrication plant in Jerusalem, which will employ 600 people from mid-1984. The Israeli subsidiary of another US corporation, Motorola, has during the past two decades increased production from \$500,000 in 1964 to \$65 million in 1982. Moreover, 42 per cent of its exports (worth \$30 million in 1982) consisted of products developed in Israel.

Eliezer Livnat, of Motorola-Israel, offers the experience of his company as proof that "links with a multinational firm do not preclude innovative research and development work by its local subsidiary". He adds that access to the sophisticated technology of an overseas enterprise has helped, especially by providing a ready-made worldwide marketing network, and argues that an overseas company is unlikely

to close down its Israeli operation so long as it remains efficient and competitive.

Some foreign companies have set up special research units in Israel because skilled manpower is considerably cheaper than in most parts of the Western world. Gelman Sciences Inc. of Ann Arbor, Michigan, for example, maintains such a unit, called Membrane Filtration Technology (MFT), at the Kiryat Weizmann science-based industrial park in Rehovot, and its head, Dr Gerald Tanny, believes that this kind of enterprise is ideal for Israel, which has a reservoir of scientific and technological talent but limited production facilities and a dearth of natural resources.

Tanny believes that Israel's research and development should be concentrated in three areas, electron optics, genetic engineering and chemicals. Marketing, in any case, should initially be handled, he says, by overseas companies "because Israel's markets are overseas".

This view is challenged by Sandy Brown, a US-trained engineer in charge of marketing for Omikron, a Kiryat Weizmann company producing pace-makers and working on other artificial implantable organs. He suggests that Israelis should follow the example set by the Japanese in the early 1950s when, in order to penetrate international markets, a large number of companies combined their marketing efforts. "And when Israeli firms get as big as Sony or Mitsubishi", he adds with a wry smile, "they can market on their own".

Nechemia Meyers

Health and smoking

British physicians lose patience

THE British Royal College of Physicians roundly condemns the promotional activities of British and American tobacco companies in a report* published last week on the effects of smoking on health. The college also laments the failure "through apathy and vested interest" of successive British governments to implement most of its previous recommendations.

The report, the college's fourth on the subject, was produced by a committee under the chairmanship of its immediate past president, and the government's chief medical officer in 1973-77, Sir Douglas Black, who has now also been asked by the government to investigate the high incidence of leukaemia in parts of West Cumbria. The report says that, for all the main tobacco-related diseases (lung cancer, chronic obstructive lung disease and various cardiovascular conditions), the chief question is no longer whether tobacco causes the disease but how to avoid these effects. The annual number of deaths due to smoking in Britain is put at not less than 100,000 — about 16 times more than are killed on the roads.

The tobacco industry, however, maintains a public stance that would be funny if it were not serious. A spokesman for the Tobacco Advisory Council, which represents all the British manufacturers, said "We in the tobacco industry do not accept that there is any causal connection between smoking and the so-called smoking-related diseases". Neither does the Tobacco Advisory Council accept that a ban on cigarette advertising — recommended by the Royal College — would lead to a reduction in tobacco consumption (although the council is, nevertheless, opposed to such a ban). The spokesman even that he thought Mr John Patten, the Minister for Health, shared this opinion.

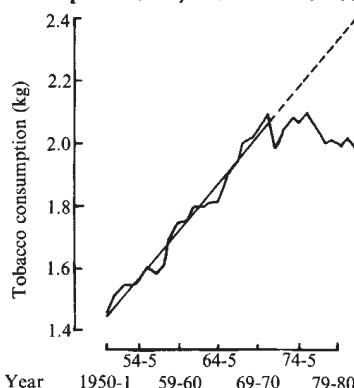
According to Mr David Simpson of Action on Smoking and Health (ASH), anyone doubting that a ban on advertising could lead to a reduction in consumption must be unfamiliar with the facts. Data from Norway quoted in the Royal College's report seem to show clearly that a ban on advertising can have a dramatic effect on consumption (see figure).

Following publication of the Royal College's report, Mr Patten said he believed that, within the constraints of a free society, government policies had proved generally effective. Although the price of cigarettes fell in real terms between 1960 and 1980, the trend has since been reversed. Mr Patten pointed out that British sales of cigarettes have declined by 20 per cent in the past four years. A major government aim is to prevent young people from starting to smoke and Mr Patten will shortly be meeting retailers' representatives to discuss how better to enforce a law forbidding the sale of tobacco to

children under 16 years of age.

The Royal College of Physicians is "not at all happy" with Mr Patten's assurances, according to its secretary Mr Michael Tibbs. Recognizing that to ask for a ban on tobacco sales would be unrealistic and even undesirable, the college wants a commitment to increase tobacco tax faster than the rate of inflation. This, it says, would give tobacco companies time to diversify their activities, as they are already starting to do. It would also prolong the government's tax revenues, which are now about £4,500 million a year. The college's president has asked for meetings with the Secretary of State for Social Services, Mr Norman Fowler, and the Chancellor of the Exchequer, Mr Nigel Lawson.

This stratagem appears to be an attempt to avoid what the Royal College claims was the fate of its previous reports on smoking: health ministers known to favour the prevention programme were moved to other departments, so that effective



Annual sales of smoking tobacco and cigarettes in Norway, as kilograms per capita (aged 15 years and over). A cigarette is assumed to contain 1 gram of tobacco. In 1970 the Norwegian Tobacco Act, which included a ban on cigarette advertisement, was first discussed. In 1975 the act became law. The regression line is fitted to sales between 1950-51 and 1969-70. Source: Directorate of Customs and Excise and Central Bureau of Statistics, Norway.

government action to phase out advertising was blocked. The Royal College describes the government's record in allowing tobacco companies to circumvent a ban on television advertising by sponsoring sports events as "particularly feeble".

Although the risk to smokers of developing lung cancer relative to that of non-smokers is higher than that for other smoking-related diseases, more excess deaths among smokers are caused by cardiovascular conditions than by cancer. Incidence of lung cancer among men in Britain is decreasing, reflecting in part the trend towards lower tar yield cigarettes. Women, however, started to reduce their consumption much more recently, and their lung cancer rates will continue to increase for some years. Although it is not

known what components of smoke cause chronic obstructive lung disease and cardiovascular disease, the Royal College recommends reductions in tar, nicotine and carbon monoxide yields.

The most alarming conclusions are those relating to tobacco consumption in developing countries. Cigarettes are vigorously promoted and smoking is increasing more rapidly than in any Western country. Tar and nicotine yields are also higher. The Royal College says "The international tobacco industry can be expected to oppose and hinder efforts to reduce smoking. In doing so, it will be directly responsible for fostering the deaths of thousands, in the twentieth century's most avoidable epidemic."

Tim Beardsley

**Health or Smoking? Follow-up Report of the Royal College of Physicians (Pitman, London, 1983).*

Royal Society row

FELLOWS of the Royal Society of London who are upset by the recent elevation to their ranks of the Prime Minister, Mrs Margaret Thatcher, are planning to press for the abolition of the statute under which she was elected.

Mrs Thatcher was made a fellow of the society last June by a special election under statute 12, which allows the society to honour non-scientists who have "rendered conspicuous service to the cause of science, or are such that their election would be a signal benefit to the society". Mrs Thatcher won the required two-thirds majority by a narrow margin. Mr David Attenborough, best known as a presenter of television films on natural history, was elected under the same rule at the same time.

Many members, however, were unhappy about having an active politician among their number — particularly one who had presided over a substantial decline in the level of public support for academic institutions. Then a newspaper suggested that the ballot to elect Mrs Thatcher had been technically in breach of the rules. Statute 12 requires that special elections should be held "not earlier than the third Ordinary Meeting" after a proposal certificate is announced. In Mrs Thatcher's case, one of the meetings was unexpectedly cancelled, so the ballot took place at the second ordinary meeting to have been held.

The society hurriedly took legal advice and its president, Sir Andrew Huxley, has now written to members assuring them that Mrs Thatcher's election stands. One consequence seems to have been that many fellows have expressed a sudden urgent wish to repeal statute 12 — although Mrs Thatcher's name was not mentioned in this context. Sir Andrew, apparently concerned that a hurried decision might nevertheless be considered to slight recently elected members, pleaded that fellows should discuss and consult on the matter over the next 12 months.

Tim Beardsley

Great Barrier Reef

Marine park complete (almost)

Canberra

By a few strokes of the cartographer's pen, virtually the whole of the Great Barrier Reef is now a marine park and under the jurisdiction of the Great Barrier Reef Marine Park Authority, a body created in 1975 at the urging of a Royal Commission inquiring into oil drilling on the reef. An area covering 344,000 square kilometres, the reef has only recently been declared a park, following an agreement between Queensland and the federal government, although it was placed on the World Heritage List in 1981.

The authority, comprising three members including a representative of the Queensland government, had the task of recommending to the federal government areas of the reef to be incorporated into a marine park, for which purpose it divided the reef into seven sections. With its recent acquisition of the Northern, Central and Southern sections (proclaimed park on 31 August), and the Townsville and Inshore Southern section (30 October), that responsibility is now discharged. But the abrupt enlargement of the reef area the authority controls, from 14 per cent to 98.5 per cent since the change of government earlier this year, will strain resources. The authority says it will need 180 extra people simply to monitor the reef from the air.

The largest system of corals and associated life, the reef stretches for almost 2,000 kilometres in a complex maze of 2,500 individual reefs and boasts at least 24 bird species, 6 species of turtle, 1,500 species of fish, 300 species of hard coral

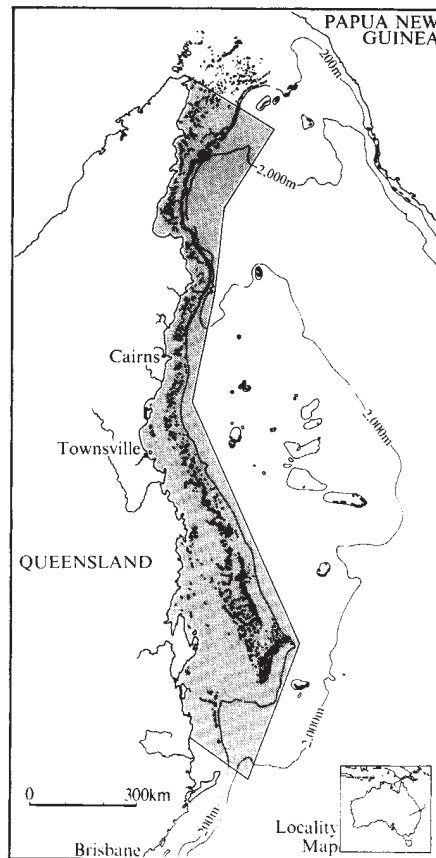
and 4,000 species of mollusc.

The park's northern boundary is latitude 10°41' S, north of which is territory disputed between Papua New Guinea and Australia. On the west, the park boundary touches the Queensland coast at the low water mark only when, as spelt out by the Minister of Environment, Mr Barry Cohen, in the House of Representatives on 2 November, there are special circumstances, such as fringing or in-shore reefs, adjacent on-shore national parks, critical habitats for endangered species, such as dugong feeding or turtle nesting areas, or areas important to the conservation of species of the reef.

Heavily-used tourist areas are also included within the park, but in general, ports and waters adjacent to industrial, urban or agricultural land are excluded.

The Queensland government was at first opposed to the park reaching the low water mark and even now would prefer a boundary some miles offshore. A decision taken by the Fraser government in 1981 to extend the Cairns section to the low water mark, against the wishes of the Queensland government, was the turning-point in the development of the park.

The administration of the park could not be more complex. Initial capital works will be paid for by the federal government, but subsequently the two governments will share expenditure on capital and operational costs equally. The authority will conduct and coordinate research, all of which is contracted out to institutions such as the Commonwealth Scientific and



The shading shows the Great Barrier Reef Region as defined in the 1975 act.

Industrial Organization and the Australian Institute of Marine Science, while the day-to-day running of the park is the responsibility of officers of Queensland's National Parks and Wildlife Service.

The Swiss-cheese park is dotted with islands belonging to the Queensland government, many of which are state national parks. Almost Kafkaesque in absurdity, these state parks end at the high water mark and since the marine park ends at the low water mark, special provisions have been made for the intertidal regions, which will be administered jointly by the two governments. A ministerial committee representing both state and federal governments coordinates policies on reef matters.

The authority has been able to acquire such a large area relatively quickly only because of the permissiveness of its writ. The marine park differs from most national parks in that most present activities will be accommodated. The only restrictions specified by the Great Barrier Reef Marine Park are on oil drilling and mining, but after an area has been part of its park for some time, zoning plans will be drawn up with public participation and may prohibit the collection of specimens or fishing in selected zones. Nevertheless, an activity in which substantial investment has been made is rarely prohibited, as zoning in Capricornia, the first section to be declared park, has demonstrated. As a spokesman for the authority explained, "proclaiming the park is the easy part, zoning it will be difficult".

Vimala Sarma

Aldabra's new status

THE Seychelles Islands Foundation is celebrating Aldabra's designation by the United Nations Educational, Scientific and Cultural Organization (UNESCO) as a World Heritage Site with a photographic exhibition at the Commonwealth Institute in London. Aldabra, an uninhabited raised coral atoll in the Indian Ocean, became a rallying cry for ecologists in the 1960s after the British Ministry of Defence surveyed the island as a possible site for a military airfield. The plans were eventually dropped and since 1976 the island has been part of the Republic of the Seychelles.

Aldabra is most famous for its large population of giant tortoises (it is probably the only terrestrial ecosystem dominated by a reptilian herbivore) but also supports many bird and plant species. As the ecology of the island is comparatively undisturbed by man and controlled largely by the climate, Aldabra is a unique natural laboratory. Fortunately, difficulty of access and inhospitable terrain deter tourists.

The Seychelles Government is keen to conserve Aldabra for scientific study, and

in 1980 established the Seychelles Islands Foundation, an independent charity, to manage it. The foundation maintains on the island a research station built by the British Royal Society, which meets half of the £50,000 annual cost. Contributions to the Seychelles Islands Foundation, which is dependent on funds it raises itself, are handled by the Royal Society in London.

Tim Beardsley



The white-throated rail, found only on Aldabra

Bacterial life in space

SIR — Your news item on the recent discussion meeting of the Royal Astronomical Society accurately reports the impasse between the two contending theories of interstellar grains. However, we would like to correct and clarify some technical points.

(1) H. Kroto's claim that there would be many ways of explaining the detailed profile of the 3.4 micrometre absorption feature was not substantiated by calculations based on any calibrated laboratory spectrum. No detailed fit on a satisfactorily large scale comparable with ours¹ was shown at this meeting. Kroto's further claim that we had underestimated error bars on the astronomical observations was based on a hasty comparison between two graphs with different marked ordinate scales. When proper account is taken of these different ordinate scales there is no discrepancy. The statement that there is a wavelength shift between the peak absorption in bacteria near 3.4 micrometres and the astronomical observations was also incorrect. There is such a discrepancy when early astronomical data are used², but this was removed by later observations made independently of our laboratory measurements^{3,4}. The latest data show a precise coincidence with the bacterial absorption near 3.4 micrometres and generally throughout the 2.9–3.9 micrometre waveband.

(2) The statements relating to the 2,800 Å interstellar absorption due to amino acids (or lack of it) are worthy of comment. We did not raise this matter at the meeting as we felt it was *sub judice* because of objections that the IUE spectra were saturated near 2,800 Å. This may be so, but the internal evidence from the distribution of noise peaks did not indicate this, and when our student L.M. Karim obtained these spectra from Culham, he was not warned of a saturation problem. In any event the detection or otherwise of this band is peripheral to the identity of the grains.

(3) Neither side in the debate conceded any ground on the question of whether grains were biologic or not. However, both sides seem to have converged on the conclusion that grains must have a major organic component with at least 30 per cent of C in grains tied up in CH bonds. Ironically, precisely this situation was being strongly denied in your columns less than 5 years ago^{5,6}. We might possibly be justified in claiming this agreement with our own earlier established position⁷⁻⁹ as a modest measure of success.

F. HOYLE

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SIR — Philip Campbell's excellent report (*Nature* 17 November, p.218) of the Royal Astronomical Society Discussion Meeting "Are interstellar grains bacteria?" claimed that "at the end, none of the protagonists had conceded ground". Yet the article also reports that Hoyle and Wickramasinghe do not now claim that interstellar bacteria are alive. In his summary, Hoyle was forced into this position by the results of laboratory work by Greenberg at Leiden on the survival of bacteria subject to UV irradiation.

This claim by Hoyle is clearly the last of many evolutionary steps in the "bacteria" hypothesis. For, if the bacteria are no longer alive, they cannot restore themselves after chemical or radiative damage. Thus, within a few thousand years, they lose all their desirable optical properties, and cannot, therefore, supply the observed interstellar spectral features.

The *Nature* report did not mention the difficulties arising from interstellar depletion studies, as discussed at the meeting. Briefly, if the Sun gives the standard relative abundances of the elements in the interstellar medium, then the requirements of the bacteria model exceed the availability of both oxygen and phosphorus. Those attending the meeting were also impressed by photographs, presented by McDonnell, of recovered interplanetary dust in which silicate crystals were clearly visible. If siliceous material is responsible for the 10 micrometre feature observed in emission and absorption in the interstellar medium, then "bacteria" are again redundant, and in any case do not supply a good fit to the observed profile, as Butchart and Whittet have shown.

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Nobel Assembly

SIR — In connection with your article "Nobel Prize to Barbara McClintock", *Nature* 13 October, p.575, I should like to point out that the Nobel Prize in Physiology or Medicine is awarded by the Nobel Assembly at the Karolinska Institute in Stockholm and not by the Swedish Academy.

JAN LINDSTEN

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The Nobel Assembly,
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S-104 01 Stockholm, Sweden

Yellow rain

SIR — The correspondence in *Nature* on the subject of "yellow rain" reminds me of an observation made by Charles Darwin. A "yellow rain" was noted more than a century ago in the *Gardeners' Chronicle and Agricultural Gazette*, no.29, 18 July 1863, p.675. Paraphrased below are the contents of this paper and a letter by Darwin which was incorporated into the article. This information was gathered from Vol.II of *The Collected Papers of Charles Darwin*, edited by P. H. Barrett.

"Mr Charles Darwin forwarded a letter regarding the occurrence of a very slight shower lasting hardly more than a minute on the morning of July 2 about 10 o'clock. Emma Darwin gathered flowers shortly after the shower and noticed that the drops of water appeared yellowish and the white roses were all spotted and stained. Microscopic examination revealed brown spherical bodies 1/1,000 of an inch in diameter and covered with short, conical transparent spines, and other smaller smooth colourless sacs about 4/7,000 of an inch in diameter. The water swarmed with elongated moving atoms only just visible. The petals on drying seemed to contain an impalpable matter the colour of rust of iron. Mr Darwin suggests that perhaps the Rev M.J. Berkeley might tell us what the larger spherical bodies are which fell this day by myriads from the sky carried by some distant whirlwind.

"Some days after the event another individual examined a leaf spotted with yellow dusty patches finding grains of Fir pollen and a few spores of Fungi along with spots from another unidentified plant but the principal constituent consisted of a slightly ferruginous apparently siliceous dust. This observer states that Fir pollen is often carried by the wind and deposited by rain on leaves. The pollen observed bore a strong resemblance to the grains of some Malvaceous plant but *Malva sylvestris* is the only species which could supply sufficient quantities of pollen to tinge the rain with a yellow tint; however, that species of pollen is smaller than the particles Darwin observed. The conclusion drawn was that it is quite astonishing what a multitude of bodies are carried about by the wind in the form of dust."

BRAYDON C. GUILD

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Imperialist threat?

SIR — I was disturbed when I first saw M.G. Audley-Charles's "Reconstruction of eastern Gondwanaland" (*Nature* 3 November, p.48). An engineering project of this magnitude, unprecedented in my knowledge, could only be financed and undertaken by a consortium of Western nations. Yet, the land masses involved are, for the most part, of the "Third World". Is this an appropriate use of resources and technology? And by what right do we disrupt other continents? Imagine my relief when I learned it was a theoretical reconstruction.

JOHN T. DURKIN

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Paying for high-energy physics

The British research establishment is agonising about the cost of contributing to overseas research establishments. Soon it may be worrying about continued membership.

THE reasons why the British grant-making research councils have these past few months been complaining about the cost of their continued membership of international research organizations are, outwardly at least, largely accidental. Membership of an overseas club such as the European Organization for Nuclear Research (CERN) at Geneva requires that membership fees should be paid in the currency of the place where the collaborative laboratory is sited, and where it must pay its bills. It is natural enough that when sterling has been falling relative to the US dollar but when the Swiss franc (as always) has been stable, the burden of membership in such an organization should seem especially onerous. There are, however, deeper questions that will not go away even if sterling miraculously recovers on the international exchanges, which apply also to the other member states of CERN and which are certain in the years ahead to raise the question of how a balance should be struck between what is sometimes called "big science" and the rest.

The case for CERN has until recently seemed without flaw. Quite apart from its utility as a means of discovery in particle physics, CERN is rightly held up as one of the first tangible demonstrations that international collaboration between partners of roughly equal technical competence can be made to work. Legally, CERN exists as a consequence of a treaty by which its member governments have bound themselves to continue to collaborate, but under the terms of which the management of the enterprise and the planning of new projects rests where it belongs, in the hands of the technical directorate and its technical advisory committees. While relationships between the laboratory and member governments have from time to time been tense, for example when new accelerators have been planned, the laboratory has benefited from its members' knowledge that there is no such thing as partial membership.

Even so, CERN's development has not been nearly as orderly as it might have been. Especially in the past decade, CERN has been able to build new accelerators only by promising its members that this would be done within the confines of a substantially static budget, adjusted only modestly for inflation, Swiss-style. As a consequence, it is possible to build a new accelerator only by shutting down one still in operation.

This is what will happen at Geneva four years or so from now, when the electron-positron collider called LEP may be in action, and when it will be necessary to cease using the super-proton synchrotron now in service which has allowed CERN to boast of the discovery of the particles called intermediate vector bosons. Nobody knows whether 1987 will be the best time to put the machine out to grass.

In the 1960s, Professor Victor Weisskopf urged that governments would profit from membership of CERN only if they were prepared also to spend generously on smaller machines operated nationally. Some, such as France and West Germany, were inclined to do so. Others, such as Britain, have long seen membership of CERN as an alternative, and with luck a cheaper alternative, to national support for high-energy physics.

The years ahead are likely to see further backsliding, and the British case typifies what may happen elsewhere. The research councils are under pressure not because their budgets have been cut but because demands on their resources have increased. If, two decades ago, the question was whether it was possible to support a national programme in high-energy physics while remaining a member of CERN, the question now — or soon — must be whether high-energy physics as such can be afforded.

This is the starting point for the case against CERN. The budget for the current year amounts to SF 680 million, more than two-thirds of the estimated cost of the LEP accelerator now being built. This is a vivid illustration of how the cost of high-energy physics lies not so much in the huge machines themselves as in keeping them serviced and supplied with electricity. And member governments or their grant-making agencies know it is pointless to pay membership fees and then fail to provide potential users with the funds needed to build equipment.

But has not CERN amply justified itself this year by the discovery of the two vector bosons (electrically charged and neutral) predicted by the Salam-Weinberg theory of weak interactions? In increasingly parsimonious Britain, even that argument is not persuasive. For the theory may equally have been held to have been confirmed by what was learned (again at CERN) in the 1970s about the neutral currents also predicted. And how can the importance of the discovery of a set of new particles of

matter be evaluated in Swiss francs per annum, however many it may cost? Even this question, unfortunately, when asked rhetorically, is no longer unanswerable.

The argument that CERN nevertheless trains young physicists in techniques not elsewhere practised, true though it may be, is equally no longer compelling. Circumstances may change if free-electron lasers turn out to have an easier and brighter future than can now be foreseen, but CERN's boast that in 1982 its facilities were used by more than 2,500 people from more than a hundred universities prompts the question what they would have been doing otherwise.

Meanwhile, there are grounds for believing that the interest in high-energy physics in comparatively small academic communities such as the British has indeed distorted the pattern of research. Ironic thought it may seem, Professor John Kingman, the chairman of the Science and Engineering Research Council which pays the British subscription to CERN, confessed to last month's general assembly of the European Science Foundation that too little is being spent on low-energy nuclear physics. And most of the innovations that have led to novel ways of gathering evidence indirectly about high-energy phenomena appear not to have been wrung by ingenuity from the ashes of severe deprivation but, instead, to have been devised in the United States.

The moral in all this is not that Britain should promptly pull out of CERN but that such a decision may have to be considered more seriously than hitherto by the end of the decade. By then, no doubt, LEP will be working well — and its designers will properly want to build a larger machine. By then, there may also be some experience with the next larger accelerator in the United States, and some chance of telling whether diminishing returns are in sight.

But would not a decision to pull out be in effect a decision that there are some natural phenomena whose true explanation will never be understood? Not necessarily. A thoroughly international and not just European collaboration might be made to work, and to cheapen the cost of participation. So might entirely new models for supporting research in this field, something along the lines of an international research foundation. And the time may come when cheap theory will be more confident of itself. By the end of this decade, Britain will not be alone in asking such questions. □

Low-temperature physics

Knudsen effects in liquid helium

from Peter McClintock

KNUDSEN EFFECTS, the seemingly anomalous behaviour seen in a rarified gas when the molecular mean free path becomes comparable with the size of the container, were first investigated systematically just after the turn of the century. Subsequently, closely analogous phenomena were discovered in other systems, including some which, at first sight, appear to be anything but rarified. Recent work at millikelvin temperatures in a number of laboratories has now added another two interesting examples to the list. Parpia and Rhodes of Texas A & M University have presented the clearest evidence yet for Knudsen effects in pure liquid ^3He , including the observation of a so-called Knudsen minimum in the reciprocal viscosity (*Physical Review Letters* **51**, 805; 1983). Their work complements very nicely an investigation of mean free path effects in liquid ^3He - ^4He isotopic mixtures, reported just a few months earlier by Guénault, Keith, Kennedy and Pickett (*Physical Review Letters* **50**, 522; 1983) of the University of Lancaster.

It seems, at first sight, that a particle's mean free path — the average distance it travels freely before colliding with another one — cannot possibly be much greater than the average inter-particle spacing, which is small in a relatively dense system such as liquid ^3He . Although such a viewpoint is certainly in accord with common sense, it ignores some quantum mechanical considerations that turn out to be of crucial importance. The ^3He atom, with half-integral intrinsic spin, is an example of what is called a fermion and is therefore subject to the Pauli exclusion principle. In other words (and in close analogy to the case of the electrons in atoms, which are also fermions) no two ^3He atoms are ever permitted to be in exactly the same state in the same system. It is scarcely surprising that, where a large number of ^3He atoms share a container with each other, this restriction results in some curious effects. This is especially so at very low temperatures, when the assembly tries to get into the lowest energy state possible for it.

The ^3He atoms are only permitted to occupy certain definite quantised kinetic energy states. At $T=0$, the absolute zero of temperature (if we ignore, for now, the existence of the superfluid transition), all of these states up to a certain characteristic energy (the Fermi energy, E_F) will be occupied; but those of higher energies will all be empty. Thus, surprisingly, even at $T=0$, a collection of ^3He atoms will still possess a great deal of kinetic energy. As T is raised above zero, some of the ^3He atoms occupying states immediately below E_F get

promoted to previously empty states just above it, but the vast majority of the low-lying states remain occupied just as before. This results in some extremely interesting and important consequences for the mean free path of the ^3He atoms. A pair of ^3He atoms can only collide with each other if there are two empty states of appropriate energy for them to occupy after the collision. For most of the atoms, however, all states that are suitably close in energy are already occupied thus implying, in effect, that collisions cannot occur. The only atoms that can collide with each other

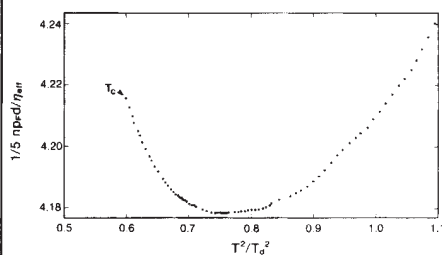


Fig. 1 The Knudsen minimum in liquid ^3He , as measured by Parpia and Rhodes. The ordinate values are proportional to the reciprocal of the effective viscosity, η_{eff} , and the abscissa is proportional to the square of the absolute temperature, T^2 .

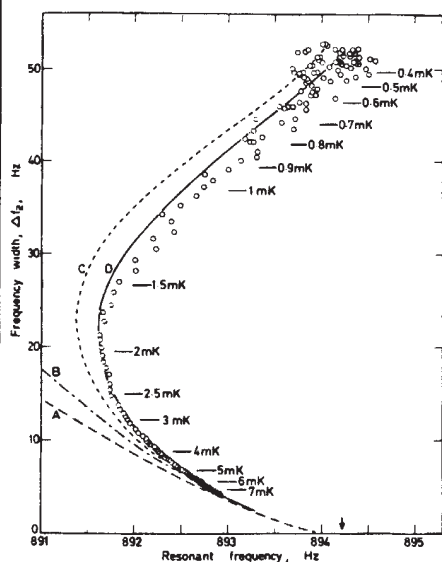


Fig. 2 Knudsen effects in a liquid $^3\text{He}/^4\text{He}$ solution, as measured by Guénault et al. The abscissa plots the resonant frequency of a wire vibrating in the liquid, and the ordinate shows the corresponding frequency width of the resonance. Temperatures are as indicated by horizontal dashes. Curve A is the behaviour predicted by classical (Stokes Law) viscosity theory; curve B represents an attempt by Jensen et al. to make some explicit allowance for incipient Knudsen effects; curves C and D, calculated on the basis of ideas due to Carless et al. clearly give a much better fit to the experimental data.

are the small minority whose energies lie within the mixed region of filled and empty states near E_F , and the mean free path is consequently a great deal longer than the interatomic separation. Because the region of partially-filled states decreases in width with decreasing temperature, correspondingly fewer collisions will then be able to occur and so the mean free path will increase as the temperature goes down. In fact, it is readily shown that the mean free path should vary with the inverse square of the temperature.

The viscosity, or 'thickness', of a fluid is directly proportional to the mean free path of its constituent particles, and it is therefore to be expected that the viscosity of liquid ^3He will also increase with falling T , so as to be proportional to $1/T^2$. Experiments have shown that this is indeed the case over a wide range of temperatures. The interesting question arises, however, as to what will happen when T has fallen to such a low value that the mean free path has grown to become comparable with the dimensions of the container itself, a situation analogous to the Knudsen regime for a rarified gas. Parpia and Rhodes have investigated the problem experimentally by measuring the viscosity of liquid ^3He in an extremely narrow annular channel, only 45 μm in height. Their results, plotted in Fig. 1 in terms of dimensionless variables, show that the viscosity does not, in fact, go on increasing indefinitely as the temperature is reduced. Rather, it reaches a maximum value (corresponding to the minimum in the reciprocal viscosity plot) and then starts to decrease again. A detailed comparison with theoretical predictions by Jensen, Smith, Wölffe, Nagai and Bisgaard of the Ørsted Institute (*J. Low Temp. Phys.* **41**, 473; 1980) shows reasonable qualitative agreement, but significant quantitative deviations from what was expected. Unfortunately, it was impossible to extend the experiments to lower temperatures, in order to penetrate further into the Knudsen region, because of the superfluid phase transition in liquid ^3He (T_c in Fig 1), which opens up an energy gap at E_F and introduces a number of other complications.

It turns out, however, that the basic physics of a Fermi fluid in the Knudsen regime can be revealed in considerably greater detail by studying, not pure liquid ^3He , but a $^3\text{He}/^4\text{He}$ isotopic mixture. Up to ten per cent of ^3He can remain dissolved in superfluid ^4He (the common isotope of helium) even at the very lowest temperatures. Near 1 mK, the presence of the ^4He solvent can for most purposes be ignored, its role being that of a 'mechanical vacuum', rather like a nineteenth century aether, in which the ^3He atoms can move freely. The two special advantages of an $^3\text{He}/^4\text{He}$ solution are, first, that the ^3He mean free path at any given temperature is over ten times longer than in pure ^3He and, secondly, that no superfluid transition occurs among the ^3He atoms (or, at least,

none has been found to date). Knudsen effects should therefore be much easier to observe and interpret.

Guénault *et al.* have measured the damping of a vibrating wire immersed in a saturated $^3\text{He}/^4\text{He}$ solution down to the unprecedentedly low temperature of 0.3 mK. They were able to make a continuous set of measurements between the two extremes of very long, and very short, mean free path (as compared to the 124 μm diameter of the wire), with results as plotted in Fig. 2. The abscissa plots the resonant frequency of the wire and shows how this quantity varied with temperature. Its departure from the value measured in a vacuum (arrowed) gives an indication of the change in the effective mass of the wire, and hence of how much fluid is being dragged to and fro as it vibrates. The ordinate axis, which plots the corresponding width of the resonance, provides a direct measure of the drag force on the wire as it moves through the liquid.

Several features of these data are worthy of comment. As T is reduced below 10 mK, the drag and the quantity of entrained fluid at first increase together, just as one would expect if the viscosity were increasing. Below about 2 mK, however, the amount of entrained fluid starts to decrease again, whereas the drag force continues to grow. Under these peculiar circumstances, the concept of a viscosity has evidently ceased to be useful. Finally, at the lowest temperature, the drag has become virtually temperature independent. It is also evident that no fluid at all is then being entrained with the wire, since the resonant frequency has returned to its vacuum value. The latter situation would appear to be analogous to the fully developed molecular flow regime in a rarified gas, where the constituent particles collide frequently with walls and other material surfaces, and practically never with each other.

It is interesting to note that the theory of Jensen *et al.* (curve B) provides only a modest improvement over the classical Stokes Law theory (curve A), which takes no account at all of Knudsen effects. The theoretical curves C and D, based on the ideas of Carless, Hall and Hook (*J. Low Temp. Phys.* 50, 583; 1983) of Manchester University, are a good deal more successful in fitting the experimental data, although they incorporate one or two assumptions which have yet to be justified in detail. In the light of the results shown in Fig. 2, the quantitative deviations of Parpia and Rhodes' pure ^3He measurements from the theory of Jensen *et al.* do not seem particularly surprising.

It is abundantly clear from these experiments that the finite mean free path effects first studied by Knudsen in classical gases at low pressures are also of crucial importance in determining the transport properties of liquid ^3He and $^3\text{He}/^4\text{He}$ solutions at millikelvin temperatures. □

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Developmental biology

Vertebrate embryos: diversity in developmental strategies

from Jonathan Cooke and John A. Webber

A fascinating conundrum is emerging for those interested in mechanisms of pattern formation in early embryonic life. It has always been assumed that the formation of the body plan in diverse embryos will prove to utilise, if not something as universal as the genetic code itself, at least differently balanced versions of a small repertoire of processes. This hope seems especially justified in embryos of animals whose anatomy suggests that they share most of their evolutionary history. However, it now appears that amphibians, whose intensive study has been defended (in funding terms) on the grounds that they are zoologically almost next door to mammals and thus ourselves, may begin development with such a different strategy that this rationale is called into question. We may now have to reconsider just what features of our own early development can appropriately be investigated in the amphibian model system, but some further research is needed first. Several recent papers and some unpublished work, on initial development in the amphibian¹⁻⁶ and in the sea squirt^{7,8}, are highly relevant.

The problem is that of how spatial patterns of committed states can reliably emerge among the early cell population of the embryo⁹. For birds and mammals it is well understood that the pattern of the embryo body itself arises relatively late, by some mechanism that cannot utilise previously specified lineages or partitioning of the egg plasm but must arise *de novo* in just one part of the by then extensive cell population.

In the amphibian, where all cells are incorporated into the body plan, it has traditionally been believed that the pattern is specified slowly and progressively across the marginal zone (the belt of material near the egg's equator), over many hours while cleavage divides the egg into some thousands of blastomeres. The reference position from which this process appeared to be controlled was termed 'the organiser'¹⁰, and was recognised to lie near a particular part of the lower edge of the marginal zone. Grafting experiments indicated that this region alone inherited its own determination, to form anterior and dorsal mesodermal parts, from the outset of development.

Two important embellishments of the traditional amphibian story must be mentioned. We now know that the organiser has its first seat well below the egg equator, in material destined to become endoderm (gut) tissue. Determinations subsequently occurring within other tissue layers are ultimately reflections of a spatial pattern of

stimuli from endodermal locations^{1,11}. The sequential transfers of such stimuli are usually thought of and modelled as diffusional processes in which levels (concentrations?) of signals are interpreted as positional cues to give pattern^{12,13}. Recently, another big step has been taken towards a modern understanding of the 'organiser' localisation in these eggs. After fertilisation but before onset of cleavage, an asymmetrical re-organisation of egg contents in relation to gravity, the point of sperm entry and the non-fluid cell cortex occurs, driven from the linked actomyosin and microtubular cytoskeletal array which becomes organised around the sperm aster¹⁻⁵.

Once true embryogenesis has got under way, development of the mammal or bird type also exhibits an organiser region (that is, Hensen's node or its mammalian equivalent), and this is 'homologous' with the amphibian one in the contribution it makes to the final anatomy. Thus the only difference in principle between bird/mammal (or indeed bony fish) development and the amphibian model has appeared to be that in the latter, the organising boundary for the development of pattern is 'given' as an initial ooplasmic localisation, whereas in the former types even this region, with its unknown but special biochemical properties as a source of signals, arises *de novo*. On the basis of all this, one might feel entitled to proceed by investigating the biochemistry of the large and accessible frog organiser region, as an origin for slowly spreading signals, to deduce a mechanism of pattern establishment that would operate also in mammals with their *de novo* arising organiser. However, here the latest, cautionary twist comes to the tale.

Slack^{14,15} has recently distinguished between embryonic specification and a later phase of commitment. Specified material or tissue has been given information leading it to construct particular parts of the body, but only in the absence of subsequent countermanning signals (for example, those caused by its experimental re-positioning within the embryo). In committed tissue, by contrast, the course of development has been effectively restricted by the signals it has already received, regardless of any future experimental disturbance. The traditional interpretation of amphibian development has been that even the specification for the normal pattern is arrived at only gradually. Thus large fragments of the embryo not including the organiser, isolated relatively late in the cleavage period, were said to dif-

ferentiate only blood-forming tissue as a kind of unactivated 'ground-state'. The reciprocal, organiser-containing fragments were held to 'regulate', giving small, harmonious and whole patterns. However, recent work, partitioning the material in similar ways but at much earlier stages, has indicated that a frequent result is the development of complementary part-patterns, each corresponding closely with what the isolated cells normally contribute to the whole (ref. 6 and unpublished work). This implies that sources for specification of each body region are appropriately distributed across the fertilised amphibian egg by onset of cleavage. It is beginning to seem as if classical workers could have done with proper anatomical reconstruction of more of their developed embryo fragments, and the cell lineage tracers that can now be used to plot the normal fates of early cleavage products.

In urochordate (sea-squirt) development, parts of the egg will still develop according to their normal fates when isolated from early stages. Recent investigations have revealed that in the egg the cytoplasm that will form the muscle regions — a major part of the body plan — carries maternally derived mRNA for muscle proteins^{7,8}. This plasm is brought into its definitive position by movements that follow fertilisation and are reminiscent of those seen in amphibians. It has not actually been suggested that these mRNAs are in themselves ooplasmic determinants, but that they correlate with them in distribution and are the nearest thing to a determinant for which we have probes.

There appears, then, in urochordates to be a type of development in which at least the mesodermal and endodermal components of the entire body are specified in outline by information throughout the egg plasm, deployed soon after fertilisation. The urochordates themselves have clear evolutionary links with primitive vertebrates, as shown in their tadpole-like larvae¹⁶, and early vertebrates in turn probably had eggs and development of an 'amphibian' sort. The great reported difference in organisation of development underlying obviously homologous anatomies — very 'mosaic' sea squirts and very 'regulative' amphibians — had always worried evolutionary-minded embryologists. But what the new work on amphibians suggests is that the problem may actually be more one of squaring their development with that of other, more regulative vertebrates.

How is a belief in the early and widespread specification of the material in amphibian embryos to be reconciled with their observed capacity to normalise the sequences and proportions of pattern parts after operative disturbances? There is no doubt that respecification (that is, change in development fate) of amphibian material can occur after re-positioning it *in embryo* or *in vitro*¹⁵. What is unclear is the extent to which this is the mechanism of

pattern stabilisation in normal development or its adjustment after disturbance. The organiser region is clearly special, but could be acting as a stable focus for the selective movement or assortment or pre-specified cells, as well as a source of information that can re-specify. Taken over all, the available evidence allows for (though it does not actually demonstrate) a substantial but hitherto unsuspected role for such selective cell movement, in the re-organisation of the mesodermal body pattern that follows surgical transplantation of the amphibian organiser region. If this indeed occurs, we would expect it to be but the dramatic extension of a recognition capacity which would be at play in stabi-

lising and sharpening the frontiers between territories in the normal pattern, as the founder cells that compose it multiply and jostle for position. The surface specialisations of pre-specified cells, that would be implied by such a capacity for sorting out, as well as the molecular 'code' for the states of specification that precede true commitment, would almost certainly be general to vertebrate embryos and thus apt for study in their largest and most accessible examples. □

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Electrophysiology

A new view of the sodium channel

from Hans Meves

ELECTROPHYSIOLOGISTS have for many years measured the time constant of inactivation of sodium channels from the decay of the inward sodium current recorded with the voltage clamp method. New work reported in this issue of *Nature* now suggests that this time-honoured practice is not after all correct: a remarkable analysis of single sodium channel behaviour by Aldrich, Corey and Stevens shows that the decay of the inward sodium current cannot be identified with the inactivation of sodium channels; rather, it appears that inactivation is a rapid process and the slow decay of the inward current is due to slow components in the opening of sodium channels. Earlier suggestions that activation may be slower, and inactivation, faster, than previously believed² are thus elegantly confirmed and a major modification made of the classical Hodgkin and Huxley³ view of the nerve impulse — at least for the mouse neuroblastoma cells that were the subject of the experiment.

Since Hodgkin and Huxley's pioneering work on squid axons, physiologists³ have measured the inward sodium currents of nerve and muscle fibres and fitted them with the Hodgkin-Huxley equations. The transient inward sodium current is the sum of the many small currents flowing through a large number of individual channels. The fast rising phase is due to the activation or opening of sodium channels, the much

slower falling phase has usually been thought to reflect the transition of the channels into an inactive (or refractory) closed state. Only recently, thanks to the development of the patch clamp technique by Neher and Sakmann⁴, has the recording of the minute currents through individual sodium channels become possible — a breakthrough comparable to the step from whole nerve action potential recording to single nerve fibre records. The results obtained with this new method have recently been summarized and discussed in an excellent review by French and Horn².

As shown by the work of Aldrich and colleagues the information obtained from single channel work is changing the existing views on sodium channels. Whereas traditionally the squid giant axon and the frog node of Ranvier have been the favourite objects for studying the biophysics of the excitable membrane, the single channel technique is most easily applied to mammalian preparations such as mouse neuroblastoma cells and rat myotubes. The new work shows that in these mammalian preparations the generally held view that activation is fast and inactivation an order of magnitude slower is no longer valid. It now seems that activation has some surprisingly slow components and inactivation some surprisingly rapid ones, resulting in a considerable overlap of the two processes. Many channels open late when

the total current is already decaying, so that the rate of decay of the total current reflects a slow component of activation and not the time course of inactivation. Fast inactivation appears to be responsible for the closing of open channels. And, as Aldrich and colleagues show, the probability that a channel closes by returning into the resting, rather than the inactive, state is very small.

What is not clear is whether these new findings on mammalian preparations reflect a difference between mammalian and other sodium channels or whether a complete change in our thinking about sodium channels in general will now become necessary. To answer this question single channel studies on frog or squid sodium channels are required. Fluctuation analysis of the sodium current reveals similar gating kinetics in rat and frog nerve⁵; this is difficult to reconcile with the idea of species differences.

One other point raised by Aldrich *et al.* needs some further investigation. They find not only that inactivation is fast but that the mean open time of the sodium channels is independent of potential, suggesting that inactivation is not voltage-dependent. However, a report at a recent conference⁶ suggests changes in the mean open time with potential (see also ref. 7). More work will be needed to resolve this difference, which is perhaps due to differences in temperature.

Single channel measurements like those reported by Aldrich and colleagues give us a much deeper insight into the function of the sodium channel than previously possible, but undoubtedly many problems still remain to be solved. The essential problem is to combine the information obtained by the different techniques (single channel recording, total current measurements, fluctuation analysis) into a coherent picture of the sodium channel. The complications which arise from the use of different techniques are best illustrated by the recent report⁸ that the single channel conductance is strongly voltage dependent! This conclusion, based on fluctuation analysis of the sodium current in squid axons (rather than on single channel recordings), suggests that the degree of opening of sodium channels is dependent on voltage and is in contradiction to the accepted view, confirmed by single channel measurements, that the sodium channel has only two conductance states — closed and open. □

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Molecular genetics

Transposable elements and plant gene structure

from John R.S. Fincham

A SYMPOSIUM under the title of 'Transposable elements and plant gene structure' took place in West Germany very recently. It was financed by the Bayer Company and held in the impressive new Bayer Pflanzenschutzzentrum; circumstances that testify to a belief in the potential commercial importance of plant genetic engineering. There was, in fact, only one contribution with a strong engineering flavour; T. Hall (Agrigenetics, Wisconsin) had introduced a bean gene coding for the seed protein phaseolin into tobacco plants by use of the *Agrobacterium* Ti plasmid. The remainder of the meeting was devoted to basic investigations of plant genomic structure and showed how rapidly the gap between plant and animal molecular genetics is narrowing.

The two apparently different themes of the symposium title are, in fact, closely intertwined. Investigation of gene structure depends on gene cloning, which has usually been carried out through the use of cDNA probes reverse-transcribed from messenger RNA. Once a gene has been cloned it can itself be used as a probe for the cloning of its own mutant derivatives, including those that have been inactivated through the insertion of mobile genetic elements. Thus any cloned gene can be used as a trap for such elements. Once a mobile DNA sequence has been so captured it can in turn be used as a probe to seek out any gene into which a similar sequence has been inserted. The latter strategy is clearly going to be very important, since it should enable the cloning of any gene, even if it is not abundantly transcribed, provided only that its inactivation has a distinct effect on the phenotype.

The new information on plant gene structure was impressive but, for the most part, unsensational. The main message was that genes of higher plants are very much like genes of higher animals. Their intervening sequences (introns) may be shorter, on the whole, than we are used to seeing in mammals and birds, but the 'consensus' sequences at the intron splicing junctions are very much the same. Furthermore, most of the plant genes sequenced so far have typical CAAT and TATA sequences upstream of their transcription startpoints, and AATAAA upstream of their transcription termini. The most notable gene on show was probably *Adh-1* of maize (coding for alcohol dehydrogenase), with its nine introns and the opportunities that it offers for selection of mutants with either loss or regain of

enzyme function (W.J. Peacock, University of Canberra; M. Freeling, University of California, Berkeley).

Several of the contributors were concerned with the complexities of regulation of gene transcription. G. Feix (University of Freiburg) showed that one of the maize zein (seed protein) genes was transcribed from two startpoints separated by nearly 1 kb. D.P. Verma (McGill University) had cloned a number of soybean genes, including that coding for leghaemoglobin, that are turned on in coordinated fashion in developing root nodules. The 5'-flanking regions of these genes will doubtless be searched for a possible common regulatory sequence.

The main excitement of the symposium was generated by information at the DNA level about the *Activator-Dissociation (Ac-Ds)* system of maize, first defined genetically by Barbara McClintock nearly 40 years ago. McClintock showed that *Ac* was an autonomously transposable element and that *Ds* elements transposed only when a copy of *Ac* was present elsewhere in the genome. Both types of element inhibited gene activity when resident at a gene locus and released gene activity when they moved away; *Ds*, at least in one of its forms, also induced chromosome breaks. In the light of what is now known about transposons in bacteria, yeast and *Drosophila* it has seemed reasonable to suppose that *Ds* elements are mutant forms of *Ac* that have lost a 'transposase' function. N. Fedoroff (Carnegie Institute, Baltimore) provided clear evidence of the *Ac-Ds* relationship in the case of the mutable allele *Ac wx^{mn9}* and its derivative *wx^{mn9}*. The former has, within the *Wx* (glucose transferase) gene, a 4.3 kb insertion, which is evidently the *Ac* element already known on genetic evidence to be present in this allele. The latter has the properties of a *Ds*-controlled allele in that it reverts to wild type only in the presence of a separate *Ac*. Fedoroff finds that it has a 4.1 kb insertion, identical to the 4.3 kb *Ac* element except for a 200 bp internal deletion.

Another *Ds*-controlled allele, *wx^{mn6}*, contains a 1.7 kb insert which appears to be derived from the 4.3 kb *Ac* through a 2.6 kb internal deletion. A 405 bp *Ds* element trapped in *Adh-1* by Peacock, and a very much larger compound element found in a mutable allele of the *Sh* (sucrose synthetase) gene by P. Starlinger (University of Köln) resemble one another and

*The symposium was held on October 1-4, 1983 at Monheim, FRG, and organized by the Plant-Protection Division of Bayer AG, in cooperation with the Max-Planck-Institut für Züchtungs-forschung, Cologne.

Fedoroff's 4.3 kb *Ac* isolate only at their ends, which all share a terminal 11 bp sequence on which the *Ac* transposase is presumed to act. In their internal sequences they are largely non-homologous both with each other and with *Ac*. The sequences unique to each *Ds* tend to be present in the genome in multiple copies, which suggests, as R.B. Flavell (Plant Breeding Institute, Cambridge) pointed out, that some of the dispersed repetitive sequences that bulk so large in many plant genomes may have been dumped in their present positions by systems such as *Ac-Ds*. How unrelated sequences come to be bracketed by *Ds* terminal inverted repeats is not clear. A typical transposon-like property of *Ac* and its *Ds* relatives is that of generating short direct repeats (6 or 8 bp) at their sites of insertion. When further transposition occurs, the tandem repeat is left behind as a 'footprint' (Peacock).

Numerous other maize transposable elements remain to be investigated in molecular detail. P.A. Peterson (University of Iowa) listed seven, including McClintock's *Suppressor-Mutator (Spm)* element, which will be particularly interesting to investigate because of its penchant for switching between alternative 'phases' or 'states'. F. Salamini (University of Bergamo) described a new element, called *Bgl*, which has so far been found particularly in mutable alleles of *opaque2* (*O₂*), a gene which seems to be a regulator of the genes coding for seed proteins of the zein family. The element *Mu-1* (no connection with the bacteriophage of that name), recently discovered by D.S. Robertson, came originally from teosinte and proliferates rapidly to a high stable copy-number when crossed into maize, in which it induces many mutations (M. Freeling). A further element, perhaps of a different kind, is associated with infection by barley stripe virus (M. Freeling and S. Dallaporta, Cold Spring Harbor).

Patterns of variegation suggestive of movable elements are found in many flowering plants other than maize. A. Cornu (University of Dijon) described one such example in *Petunia hybrida*. In *Antirrhinum majus*, there is the opportunity for investigation at the DNA level since the *nivea* (chalcone synthetase) gene, of which there is a highly mutable allele *niv⁵³*, has been cloned (it proved possible to use cDNA from the parsley chalcone synthetase mRNA as a probe). H. Saedler (Max-Planck-Institut, Köln) reported his finding of two large inserted elements, one (*Tam1*, 17 kb) in *niv⁵³* and the other (*Tam2* 5.6 kb) in *niv⁴⁴*, a stable null allele. Both elements have long mutually inverted terminal repeats, those of *Tam1* being of extraordinary internal complexity with short tandem and inverted sub-repeats. Only the terminal 17 bp of these terminal repeats are common to the two elements. Curiously, the *niv⁵³/niv⁴⁴* heterozygote showed almost no instability of the *niv⁵³* allele, which suggested to Saedler that

Tam2 (which is present in the *niv⁴⁴* stock in multiple copies) might code for a repressor that inhibited both its own transposition and that of *Tam1*.

In spite of the remaining puzzles it seems likely that our appreciation of the beauty of mutational variegation in plants will soon

be enhanced by a much deeper understanding of how it arises.

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Oncogenic intelligence

Viral oncogene permutations

from Peter Newmark

WHAT at first sight are four, but on closer inspection are only three, or perhaps even two, new oncogenes have recently been added to the twenty or so that have been discovered in tumour viruses but have their origins in normal cells (reviewed in ref. 1). Of particular interest is that each of the newly discovered oncogenes can coexist with a second oncogene in a virus. That brings to three the number of RNA tumour viruses in which two distinct oncogenes cooperate in a way that superficially resembles the cooperation of pairs of oncogenes to bring about the transformation of primary human fibroblasts in culture (see these columns 306, 582).

One of the new oncogenes, described by two groups in last week's *Nature*^{2,3} has been christened *ets*, an acronym for the E26 virus in which it has been found. Like AMV, another RNA tumour virus that causes myeloblastosis in chickens, E26 contains the *myb* oncogene. Unlike AMV, E26 can cause erythroblastosis, probably due to the additional presence of the *ets* oncogene. The best evidence that *ets* is completely distinct from *myb* is that RNA transcripts from the cellular progenitor of *ets* do not contain *myb* sequences³; the best characterization of the viral *ets* oncogene is its complete sequence².

The other three — or perhaps two, or even one — new oncogenes are known as *mil*⁴, *mht*⁵ and *raf*⁶. The first two names are synonyms for a newly described oncogene of Mill Hill 2 virus (hence *mil*) which is generally abbreviated to MH2 virus (hence the acronymic *mht*). The MH2 virus, which causes both leukaemias and carcinomas of chickens, also contains the *myc* oncogene, which is found in three other, closely related viruses as well. The *mil/mht* oncogene appears to be derived from a completely different cellular gene than does *myc*; how, or even whether, the two interact to produce the carcinomas and leukaemias remains to be determined.

The *raf* oncogene is the transforming gene of 3611-MSV, a murine RNA tumour virus that induces fibrosarcomas in newborn mice⁶. When isolated, the *raf* oncogene had no relationship to other known oncogenes. But since then, *mil/mht* has been characterized and in a paper soon to be published in *Nature*⁷, Jansen *et al.* establish that *raf* and *mil/mht* are closely

related. Quite how closely will only be clear when sequences of the two viral oncogenes and their cellular progenitors become available but on present evidence Jansen *et al.* are confident that they arise from equivalent murine and avian genes.

It is not unprecedented for different tumour viruses to pick up equivalent cellular genes from different species. Three different feline sarcoma viruses have oncogenes similar to those of an avian, a feline and a murine retrovirus (see ref. 8). Nor is it unprecedented for two separate oncogenes to be found in one virus — the avian erythroblastosis virus was already known to contain both *erbA* and *erbB* viral oncogenes. But now that neither phenomenon is confined to a single case, the questions each raises become more interesting.

As to why the same gene should be picked by different viruses, the probable answer is that there is a rather small number of cellular genes that can be turned into oncogenes and that the documented list is nearing completion, limiting the more difficult problem of discovering the function of the products of the genes. That problem has probably been simplified by the classification of different oncogenes into two groups that cooperate to transform primary fibroblasts *in vitro*. It will now be important to establish whether the cooperation of pairs of oncogenes in viruses is a similar enough phenomenon to serve as a good model. There is also the fascinating question of how sequences from two distinct cellular loci come together in a virus — are they, perhaps, first juxtaposed by translocation in the cell?

Peter Newmark is Deputy Editor of *Nature*.

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Cold Spring Harbor meeting

Building new vaccines

from Nigel Williams

HEPATITIS B virus may be one of the first viruses to come under large-scale attack from a synthetic subunit vaccine and, if an acceptable way of boosting the immune response can be found, many more viruses face a similar challenge. One unorthodox way of tackling this problem may be to use recombinant vaccinia virus, a descendant of Jenner's original protocol against smallpox, as a vector. With the focus on viruses, these were some of the points arising from the first of what promises to be an annual series of meetings to consider the modern approach of vaccines.*

The modern approach is dominated by efforts to produce synthetic subunit vaccines which, in the long term, may be able to overcome problems of contamination and storage often found with conventional vaccines. Also, viruses for which there are no conventional vaccines can be tackled. Because it appears that only a restricted number of sites on the viral surface are important targets for neutralizing antibodies, the aim is to produce synthetic analogues of these regions by expression of viral proteins in recombinant heterologous cells or by chemical synthesis of short peptides. If such subunits retain the configuration found normally on the viral surface and can induce a specific immune response, the hope is that they can form the basis of safe and effective vaccines.

The appeal of this approach was reflected by the number of groups reporting work on a wide range of animal and human viruses. While many have obtained antibodies to synthetic products that cross-react with the intact virus, this is only the first step — a potential vaccine must stimulate an antibody response strong enough to protect the animal from subsequent infection. It is clear that this is a major challenge.

Hepatitis B virus is of particular interest because no conventional mass vaccine exists against this virus — the cause of widespread and serious human disease — but a natural, highly immunogenic 'subunit' can be found in the serum of chronically infected individuals. This consists of particles containing aggregated viral surface antigen (HBsAg) molecules in both glycosylated and non-glycosylated forms. Purification of these particles from serum has formed the basis of a small-scale but successful vaccine by Merck, Sharp and Dohme. It is the proven potency of these particles (and their very limited supply and high cost) that has been the impetus for obtaining expression of

recombinant HBsAg DNA in cell vectors.

Valenzuela *et al.*¹ and subsequently others identified the part of the hepatitis B genome encoding the 226 amino acids of the HBsAg by cloning viral DNA in *Escherichia coli*. The use of *E. coli* for expression however, has been difficult; but using yeast cells as vectors, W.N. Burnette (Amgen) and D.E. Wampler (Merck, Sharp and Dohme) were among those reporting successful expression. The molecule is not glycosylated or secreted but forms aggregated particles in yeast cells which appear similar to those recovered from serum. The yeast derived particles seem as potent as the plasma derived particles in stimulating antibodies; indeed, Wampler reported that the Merck, Sharp and Dohme product, purified by immune affinity, is about to undergo clinical trials.

Since publication of the first nucleotide sequence of HBsAg, several groups have also produced synthetic peptides corresponding to specific regions of the molecule. So far the ability of such peptides to induce antibodies that cross-react with native HBsAg has been surprisingly good but the strength of the response has been variable and difficult to predict. However R.A. Lerner (Scripps Clinic) has encouraging results with a peptide of 38 amino acids in length spanning a relatively hydrophilic region of HBsAg. In a trial on three chimpanzees one was protected, one developed infection without disease and one developed acute infection when subsequently challenged with HBsAg. But a problem with this and many other peptides is that a carrier protein and adjuvant are needed to boost the immune response; the majority of these agents are unacceptable for use in man.

Encouraging for a different reason is the production (E. Wimmer, SUNY Stony Brook) of several peptides representing regions of the poliovirus (type 1) capsid protein, VP1³. Although only one peptide was able to induce neutralizing antibodies in rabbits, when attached to a carrier protein and injected with adjuvant, all the peptides were able to prime the immune response so that the animals responded vigorously to a subsequent challenge with whole virus vaccine. It may be that priming an immune response is sufficient to protect an individual against viruses with a long incubation time but again, the carrier/adjuvant system used puts human use some way off.

G.R. Dreesman (Baylor College, Houston) described one way that may overcome the need for a carrier protein; he has introduced a cross-linking disulphide bond into a peptide, 16 amino acids in length, corresponding to a region of

HBsAg². This cyclic form of the peptide gave a much better antibody response than the linear form.

A quite novel way of overcoming the need for carrier proteins and adjuvants may be the use of recombinant vaccinia virus as a live vector to present heterologous virus subunits. M. Mackett (NIH) described the production of vaccinia recombinants expressing HBsAg⁴. Vaccination of three chimpanzees with these recombinants provided total protection when the animals were subsequently challenged with HBV (G.L. Smith, NIH). E. Paoletti (New York State Department of Health) reported on other vaccinia recombinants expressing the coat glycoprotein (gD) of herpes simplex virus, that were able to protect mice from subsequent, normally lethal, challenge with live virus.

These are impressive results but the use of a live recombinant vector for subunit vaccination in man is clearly controversial. Among the many potential problems are the possibly different and more dangerous tropisms of recombinant viruses and reversion of recombinants to a more virulent form. However, the enormous worldwide experience (and undoubted success) in using vaccinia virus for smallpox vaccination and the impressive immunogenicity of the recombinants suggest that it is well worth tackling these potential problems.

Although the problem of boosting the response to subunits may be overcome, the problem of antigenic lability of certain viruses remains. Foot and mouth disease virus (FMDV) appears to have a dominant neutralizing site on the capsid protein, VP1. Synthetic peptides spanning the region have been produced and are able to induce protective antibody in rabbits against the corresponding FMDV strain⁵. But FMDV is extremely labile and exists in many variant forms. D.J. Rowlands (Animal Virus Research Institute, Pirbright) described three antigenic variants within a single isolate which differ at only two amino acids within the immunogenic site. Peptides representing the immunogenic site of the different variants induce good neutralizing antibody only against the corresponding variant. The antigenic lability of FMDV suggests that a synthetic vaccine may have to comprise several different peptides tailored each time to cope with local FMDV variants.

The power of the subunit approach which allows the viral surface to be explored and the nature of specific antibody-antigen interactions examined, justifies the optimism and the growing interest in these techniques. □

Nigel Williams is assistant editor of Nature.

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*Modern Approaches to Vaccines: A meeting held at the Cold Spring Harbor Laboratory, New York, 31 August–4 September 1983.

A better value for Earth's mass

SIR — The uncertainty in the Earth's mass was recently discussed (*Nature* 302, 11; 1983) but unfortunately without considering the now most accurate value of the gravitational constant

$$G = (6.6726 \pm 0.0005) 10^{-11} \text{ m}^3 \text{ s}^{-2} \text{ kg}^{-1}$$

obtained by G.G. Luther and W.R. Towler at the US National Bureau of Standards (see *Phys. Rev. Lett.* 48, 121; 1982). By using the new value, uncertain by 7.5 parts in 100,000, and the product

$$GM_{\oplus} = 3.986005 10^{14} \text{ m}^3 \text{ s}^{-2}$$

known from satellite observations with the uncertainty of 1 part in 10^7 , we get the new value of the Earth's mass

$$M_{\oplus} = (5.9737 \pm 0.0004) 10^{24} \text{ kg}$$

This is more accurate by an order of magnitude than the quoted value of $(5.974 \pm 0.004) 10^{24} \text{ kg}$.

One interesting point arises from the measurements of Luther and Towler, who measured the period of a torsional pendulum suspended in an evacuated cylin-

drical chamber when two large spheres of tungsten placed in the air outside the chamber were brought near to the pendulum. Then the spheres were removed and the period measured again.

Luther and Towler applied a correction of approximately 1 part in 10^4 to the measured value of G to allow for the fact that when the spheres of tungsten were removed, they were replaced by spheres of air and, consequently, that the change in the force was appropriate to the differences between the mass of tungsten and the mass of air (personal communication). In fact, it can be shown (see Z.H., *Proc. 6th and 7th Conf. Czech. Physicists*, Ostrava 1979 and Prague 1981) that the gravitational force between homogenous spherical objects immersed in an infinite homogenous fluid can be obtained by substituting for the masses in the usual newtonian expression the difference between the mass of the object and that of the fluid displaced.

Corrections for the effects of buoyancy are of course routinely made in accurate

measurements of terrestrial gravitational attraction. More generally, allowing for the displacement of fluid implies that while objects both more or less dense than the immersing fluid will attract each other, those with opposite buoyancy will repel each other. The effect of the medium on the gravitational interaction between objects is thus analogous to that of a dielectric medium on the electrostatic interaction of charges (although the signs are different).

In the well-known measurement of G by Cavendish (1798), in which the mutual attractions of pairs of large and small spheres of lead were measured in air, the buoyancy correction would have amounted to two parts in 10,000 — irrelevant compared with the errors of measurement leading to an uncertainty of 1% in the value of G . The correction has been similarly negligible in all subsequent measurements of G until that of Luther and Towler, no less than 22 centuries after Archimedes.

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Relief in microscopy

SIR — The illumination systems and techniques involved in the production of the photomicrographic image are complex and radically different from those encountered in everyday life, and ambiguities and misinterpretations can arise if the brain interprets the apparently simple image in the same way as it would interpret an ordinary macroscopic photograph.

One of the most common ambiguities is illustrated on the right. The image is an electron micrograph of a metallic replica of a freeze cleaved specimen which on the left is shown in its normal orientation while the right hand photograph has been inverted.

The inversion results in a reversal of relief from cameo to intaglio. This reversal of apparent relief is simply explained by the direction of the shadows. In everyday life, shadows project downwards from objects since most lighting is overhead, so when a photograph presents shadows projecting upwards from an object the brain wrongly interprets an upward shadow from the rim of a depression as a downward shadow from a raised structure and this results in an incorrect perception of surface relief.

The misrepresentation of surface relief is not the only source of ambiguity (D.J.C., *Microscopy* 34, 533–552; 1983) and microscopists should be aware of the optical illusions which can occur in photomicrographs.

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IMAGE
UNAVAILABLE
FOR
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REASONS

A replica of a fractured surface. The correctly oriented image on the left shows a series of channels (a) which in the inverted photograph on the right appear as ridges. Other relief features show a similar relief reversal. (Reproduced with kind permission of The Upjohn Co., Kalamazoo, Michigan.)

Heat and helium in the Earth

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Questions concerning the locations of possible chemical reservoirs and heat sources are closely tied to those of convective motions in the Earth's interior. For example, if the continental crust and the atmosphere have been extracted from only an upper portion of the mantle then the convective motions in that portion of the mantle are likely to have been decoupled from any deeper motions. The predicted thermal history for an Earth with a layered mantle (separated convection between upper and lower portions) is quite different for one with convection extending throughout the whole mantle. The budgets of helium and heat in the Earth's continental crust and mantle provide important constraints on the large scale processes operating in the mantle.

THE four nuclides ^{40}K , ^{232}Th , $^{235,238}\text{U}$ are the principal sources of radiogenic heat in the Earth. In addition to liberating thermal energy these nuclides also yield ^4He (^{232}Th , $^{235,238}\text{U}$) and ^{40}Ar (^{40}K) as decay products. The isotopes of U and Th account for about three-quarters of the radiogenic heat produced today and virtually all of the ^4He : the approximate relationship is $\sim 10^{12}$ atoms $^4\text{He W}^{-1}$ contributed by U and Th decay. In addition a small amount of ^3He is produced by the reaction $^6\text{Li}(n, \alpha)^3\text{H}(-\beta)^3\text{He}$. However, this reaction accounts for a negligible portion of the ^3He escaping from the mantle through the ocean ridges and the predominant source is primordial He that has been trapped within the Earth from the time of its formation¹⁻³.

Radiogenic helium is calculated to have a production ratio of $^3\text{He}/^4\text{He} = 1 \pm 0.5 \times 10^{-8}$ in normal crustal and mantle lithologies^{4,5}. The value is close to that found in old continental areas and may be compared with the atmospheric ratio of 1.4×10^{-6} . In contrast the primordial $^3\text{He}/^4\text{He}$ ratio is presumed to be $\sim 10^{-4}$, the value measured in gas-rich meteorites⁶. Helium degassing from the Earth's mantle today has a ratio close to 10^{-5} , which presumably reflects a dilution of primordial He by radiogenic helium produced over the past 4,500 Myr.

Ocean basins

In many continental regions more than half the heat flow is attributable to radiogenic heat production in the upper 10 or so kilometres of the continental crust. This is in marked contrast to oceanic areas where the heat flux results largely from the cooling of new sea floor created at the ocean ridges. A negligible proportion arises from radioactivity within the oceanic lithosphere itself. Very high heat flow values are measured close to the ridge axis but these decrease fairly rapidly in the flank areas as material is transported away from the ridge and the effects of cooling extend more deeply. The mean heat flow for the ocean basins is $\sim 100 \text{ mW m}^{-2}$.

The helium flux from the mantle is also strongly concentrated at mid-ocean ridges (particularly fast spreading ones)^{3,7,8} but is measurable elsewhere in the ocean basins in association with intra-plate and marginal igneous activity. The mantle-derived helium degassing from ocean ridges measured in both hydrothermal vents^{3,7} and basaltic glass^{2,9} has a $^3\text{He}/^4\text{He}$ (R) ratio which exceeds the atmospheric ratio (R_a) with $6 < R/R_a < 10$. The flux of helium from the mantle through the oceans has been deduced from the integrated ^3He excess in the oceans¹⁰ and from ^3He -temperature relationships in hydrothermal vents from the East Pacific⁷. Although both approaches have produced the same result of $5 \times 10^9 \text{ atoms m}^{-2} \text{ s}^{-1}$ (Fig. 1), the first method should be an inherently more reliable approach and has a quoted uncertainty of $\pm 25\%$.

The oceanic flux may be considered as having two components, one of which is primordial with $R/R_a \approx 100$ and the other a radiogenic component which must account for $\sim 88\%$ of the total He flux. In a steady-state situation the whole mantle would require 1.5 p.p.b. (parts per 10^9) ($\text{Th}/\text{U} = 4$) to support this radiogenic flux or if only the upper mantle was involved it would require 5 p.p.b. U. This amount of U+Th could only supply a very small portion ($\sim 3 \text{ mW m}^{-2}$) of the oceanic heat flux. The igneous activity that builds the oceanic crust is largely confined to a zone a few tens of kilometres wide along the ridge crest and this is the source of He plumes recognized during the GEOSECS programme³. If it is assumed that He is efficiently stripped from the oceanic basalts and the mantle processed by basalt melt-extraction³, then the oceanic plates will be strongly depleted in He. It follows that over much of the ocean floor away from the ridge axes there will be a comparatively minor loss of mantle-derived helium, except at other sites of igneous activity. Because volcanic activity occurring along the ridges in the ocean basins is volumetrically the most important, the contribution to the total mantle He flux made by subaerial volcanics will be relatively minor (probably $< 10\%$) and so this source is not included in Fig. 1. If these assumptions are correct then the estimated radiogenic helium flux places limits on the U and Th abundance in that portion of the mantle from which He is being extracted. The steady-state contribution of heat from this source to the oceanic heat flux is estimated to be only 3 mW m^{-2} and will be even smaller if the helium is extracted less efficiently than has been assumed above. The principal source of the heat lost through the oceanic plates is therefore likely to lie deeper than the mantle tapped in the formation of mid-ocean ridge basalts (MORB) as has been suggested previously from other considerations^{11,12}.

Continents

Helium isotope compositions have been reported for many groundwaters, connate waters and rocks from regions of old continental crust (see ref. 13). Connate waters from deep wells typically have $0.01 < R/R_a < 0.05$. However, those shallower zones of groundwater circulation uncomplicated by the introduction of bomb-tritium, exhibit a wider range with $0.01 < R/R_a < 1.0$, with the older waters generally exhibiting the lowest ratios presumably because they have had longer to acquire the signature of the rocks through which they have passed. In recently volcanically-active continental areas, such as Yellowstone, thermal waters may show considerable excesses of ^3He compared with the atmospheric ratio that have been attributed to derivation from a mantle source¹⁴. Regions where such influences are observed, however, form a relatively small proportion of the continental crust.

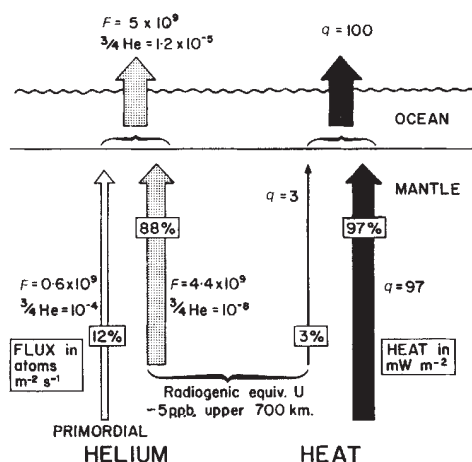


Fig. 1 The total helium flux is calculated from the $^3\text{He}/^4\text{He}$ ratio of MORB^{3,9} and the estimated ^3He flux from the mantle through the oceans¹⁰. The additional direct input of ^3He into the atmosphere is likely to be small. The primordial and radiogenic components of this flux are calculated using $^3\text{He}/^4\text{He} = 10^{-4}$ for primordial He. The estimated U content of the mantle assumes a steady state and Th/U = 3.8.

Although the He flux from the continents has not been measured directly, the rate of generation of ^4He within the continental crust may be estimated if its content of U and Th is known. It has been shown^{15,16} that within a particular geological province there is a relationship between surface heat flow and the near surface concentration of heat-producing elements, and that from heat flow-heat production pairs, it is possible to distinguish that component of the heat flow attributable to crustal radioactivity from that derived by conduction from the underlying mantle. This in turn allows the steady state rate of crustal ^4He generation to be derived. These relationships are shown in Fig. 2.

In continental areas where $R/R_a \leq 0.05$ the He flux must arise almost entirely from crustal radioactivity because this ratio is within the range expected for *in situ* production of radiogenic He. Mantle contributions ($6 < R/R_a < 30$) to the flux would be $< 1\%$ in such situations. In the stable areas where low $^3\text{He}/^4\text{He}$ ratios are found, the mantle contribution to the heat flow is also low. There appears to be a continuous gradation from old stable areas at one extreme, to those like Yellowstone at the other with the mantle contribution to both heat flow and He flux increasing to a maximum in those areas most recently active. The average U content for the continental crust, estimated from continental heat flow, is equivalent to 6 p.p.m. over the uppermost 8 km.

The treatment of heat and helium fluxes from the continental crust presupposes that their transport properties are similar at least in the upper portion where radioactivity is concentrated. Although there are presently no He flux measurements available which would allow a test of this hypothesis, the mobility of He in this portion of the continents is evident from its highly variable abundance in natural gas reservoirs and groundwaters.

The relatively abundant, radiogenic, 'continental-grown' He characterized by $R/R_a \approx 0.01$ is supplemented to a variable degree by mantle He ($R/R_a = 8-30$). Where the continental lithosphere is thick, in old stable areas, the mantle contribution is expected to be very low, reflecting both the diffusional barrier imposed by thick lithosphere and the low average abundance of He in the mantle. On the other hand in some continental areas the mantle contribution may be much enhanced (for example, in the Italian volcanic province characterized by $0.1 < R/R_a < 3$), or may even effectively mask any contributions from the continental crust (for example, Yellowstone). Therefore the $^3\text{He}/^4\text{He}$ ratio is a sensitive indicator of mantle involvement in crustal thermal activity and may provide evidence of such involvement in cases where other evidence is ambiguous or lacking.

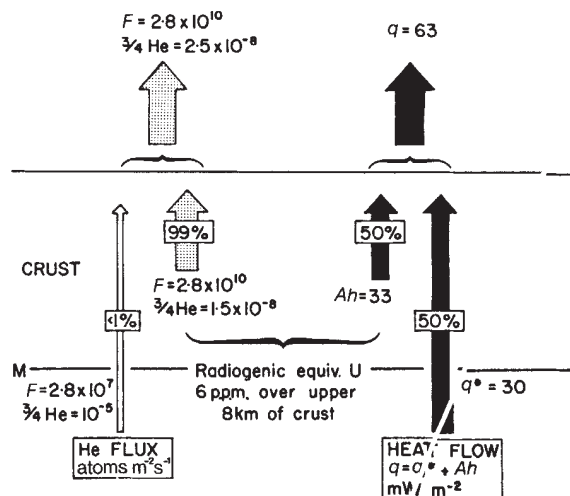


Fig. 2 An average continental heat flux (63 mW m^{-2}) is used to calculate the steady-state radiogenic He flux assuming $K/U = 10^4$, Th/U = 3.8. It has been assumed that the component of the continental flux derived from the lower crust and mantle that the remainder is radiogenic. q^* is 30 mW m^{-2} , A is the near surface radiogenic heat production and h is a characteristic scale length for its vertical distribution.

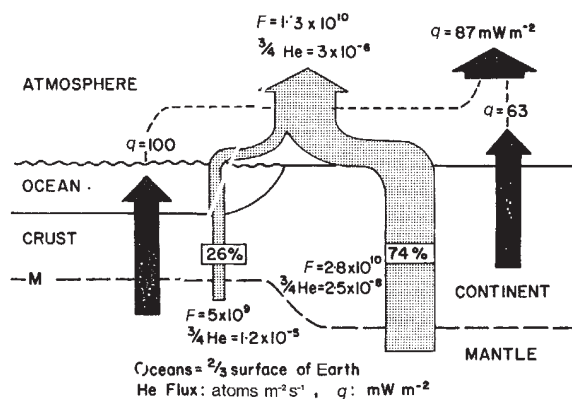


Fig. 3 Combined results summarized in Figs 1 and 2 illustrating the relative contribution of the mantle and continental crust to the total helium flux entering the atmosphere. Oceanic heat flow from ref. 28.

Mantle structure

The estimated fluxes shown in Figs 1 and 2 are combined in Fig. 3 to view the various contributions to the global He budget. About 75% of the He entering the atmosphere is derived from the continental crust and is almost entirely radiogenic in origin. The remaining 25% comes from a mantle source, of which 2-3% is primordial and the remainder also radiogenic. The amount of U and Th in the mantle required to support the oceanic radiogenic He flux would only provide $\sim 5\%$ of the mantle heat flux (Fig. 1). This observation has important implications both for the location of heat sources in the Earth and the scale of mantle convection. If all of the heat lost from the mantle is radiogenic in origin it follows that vastly more He is being generated in the Earth than is being released, or that the time constants for the loss of heat and He differ greatly.

Previously, geochemical arguments have been presented which suggested that a restricted portion of the mantle ($1/3-1/2$) has been depleted in the generation of continental crust^{11,17-19}. For a model bulk Earth with 20 p.p.b. U ($K/U = 10^4$; Th/U = 3.8)^{11,20}, the depleted portion of the mantle is estimated to contain 5 p.p.b. U which gives rise to 1.2 pW kg^{-1} , as opposed to 4.8 pW kg^{-1} for bulk Earth or undepleted mantle. The value of 5 p.p.b. U plus equivalent Th is precisely what is

required to support the estimated steady-state radiogenic He flux from the mantle. To accommodate the oceanic heat flow with only radiogenic heat sources in an Earth with 20 p.p.b. U then the time constant for heat loss must be $\sim 2,000$ Myr (ref. 20). This may be achieved if convection in the upper and lower parts of the mantle are separate, and heat transport is impeded at the boundary layer between them²¹. In this case an additional requirement of the boundary layer is that it must impede the transport of helium more effectively than that of heat.

The rare-gas isotope geochemistry of oceanic basalts is also readily accommodated if the lower part of the mantle behaves as a chemical as well as a thermal reservoir. For example, the composition of He in MORB is relatively uniform⁹ and largely seems to represent radiogenic He generated in the upper mantle diluted with $\sim 12\%$ of primordial helium presumably derived from greater depths. In Iceland and Hawaii more extreme He enrichments have been recorded with R/R_a in the range 20–30 (refs 22–26). These observations indicate derivation of a larger component of the He from a deeper source which is presumably in the lower mantle. Furthermore they suggest that He still retained in this source has $R/R_a > 30$. In addition, the lower

$^{129}\text{Xe}/^{132}\text{Xe}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ ratios in hot spot volcanics such as Hawaii compared with MORB are also most readily accommodated by a layered mantle with the lower layer behaving as a repository for primordial rare gases^{19,27}.

Overall the observations summarized above are most readily compatible with a convecting upper mantle which loses its small amount of radiogenic heat and helium efficiently, but which is isolated from a lower mantle reservoir from which $\sim 90\%$ of the heat lost through the ocean basins is derived. The removal of helium from the lower mantle is impeded at the upper boundary layer. The upper mantle may provide virtually all of the radiogenic helium lost through the oceans but only 5% or so of the heat. Because we have no constraint on the abundances of rare gases in the lower mantle, the radiogenic helium and heat budgets cannot be assessed. However, the $^3\text{He}/\text{U} + \text{Th}$ ratio must be sufficiently high to maintain $R/R_a \geq 30$. The data on hand do not rule out the possibility that a component of the mantle heat flow arises from core crystallization rather than a radiogenic heat source.

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K, U and Th in mid-ocean ridge basalt glasses and heat production, K/U and K/Rb in the mantle

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Analyses on fresh glass samples of mid-ocean ridge basalt yield a uniform ratio $K/U = 12,700 \pm 200$. In contrast, Th/U increases systematically with Th concentration. From these results we calculate an upper limit (1.5 pW kg^{-1}) and a best estimate (0.6 pW kg^{-1}) for the heat production in the depleted portion of the mantle. A new estimate is given for the terrestrial ratio of $K/Rb = 513$.

HEAT production in the Earth is governed by the abundance of the elements U, Th and K. The geochemistry of these elements is, therefore, important to the understanding of the thermal history and convective mechanism in the mantle. Given this special geochemical importance, surprisingly few efforts have been made to obtain analytical data for all three elements in mantle-derived rocks. Wasserburg *et al.*¹ showed that the terrestrial K/U ratio is $\sim 1 \times 10^4$. Recent estimates of the bulk-Earth ratio range from 0.5×10^4 to 1.4×10^4 (refs 2–13). These values are much lower than the chondritic K/U ratio of $\sim 6.3 \times 10^4$ (ref. 14) and are consistent with the well-known depletion of alkalis in the Earth¹⁵. O'Nions *et al.*¹² reviewed K/U ratios of oceanic basalts and found mean values for different oceanic

regions ranging from 0.7×10^4 to 2.1×10^4 , averaging at $\sim 1 \times 10^4$.

Estimates of the bulk-Earth Th/U ratio range from 3.6 to 4.0. These are based in part on that ratio in meteorites¹⁶ and in part on Pb isotopic data^{17,18}. The Pb isotopic data also show unequivocally that the Th/U ratio of a significant part of the mantle has decreased with time; however, the magnitude of this decrease is not well known because the isotopic abundances reflect an integrated effect of time and change in chemical composition¹⁹. The Earth's upper mantle, which is believed to produce mid-ocean ridge basalts (MORB), is severely depleted in its incompatible trace elements including K, U and Th, and mid-ocean ridge convection appears to carry more heat to the

surface than is produced at present in this part of the mantle¹². The actual depletion mechanism is not well understood, but the missing fraction of incompatible elements is believed to reside in the continental crust.

This article aims to (1) provide new data on K, U and Th abundances in depleted (N-type) MORB using only specially selected fresh glass samples, (2) combine these data with the terrestrial Ba/Rb and the chondritic Ba/U ratio to derive a new estimate of the terrestrial K/Rb ratio, (3) propose a working hypothesis for the depletion mechanism in the mantle, (4) derive a maximum estimate and a 'best' estimate for the concentrations of K, U and Th and the heat production in the MORB-source mantle.

Samples

Only volcanic glasses of dredged MORB samples were analysed. Most of these were obtained from the basalt glass collection of the Smithsonian Institution. The utmost care is required in the selection and treatment of ocean floor samples to avoid contamination by seawater; this is true not only for isotopic analyses but also, and especially so, for determining the original abundances of alkalis²⁰ and of uranium²¹. Because of this, the samples were hand crushed and picked to select only clean glass fragments with no traces of palagonite.

Data on location, tectonic setting, depth, age and major-element chemistry for most of the samples have been given by Melson *et al.*²². Locations are also shown on Fig. 1 and listed in Table 1. All samples but one are from dredges along the mid-ocean ridge; sample 367 is from a fracture zone. The three samples from location D10 represent different portions of a single pillow. Most of the samples belong to the depleted type,

also called N-type by Schilling²³, with (La/Sm)_N ratios (normalized to C1 chondrites²⁴) <0.77 (Fig. 2). Samples GS 7309-75 and EPR D-10 have higher (La/Sm)_N ratios (0.96 and 0.90 respectively), but the isotopic data suggest they belong to N-type MORB. Two samples (200, H70) have (La/Sm)_N > 1.9 and the three samples (A01, C14, C35) are differentiated with high concentrations of incompatible trace elements, Eu anomalies and partly unusual SiO₂ concentrations (C35: SiO₂ = 52.6%, C14: SiO₂ = 69.4% (ref. 22)).

Spark source mass spectrometry

Spark source mass spectrometry (SSMS) was used for the simultaneous determination of all three elements K, U and Th using an analytical technique described elsewhere²⁵. To apply multi-element isotope dilution-spark source mass spectrometry (ID-SSMS) a large batch of graphite with isotopically enriched elements was made. The pulverized sample was mixed with part of this spiked graphite and analysed in the AEI-MS 702R mass spectrometer. K was measured by ID-SSMS with a precision of about ±3%. Th and U were calibrated by relative sensitivity factors obtained from the analyses of geological standard rocks using Hf as internal standard which was determined by ID-SSMS. The precision of the Th and U concentrations is ±5% and ±7% respectively, with the exception of samples 968, 768, 973 and F83, for which the precision is lower by a factor of two.

Thermal ionization mass spectrometry

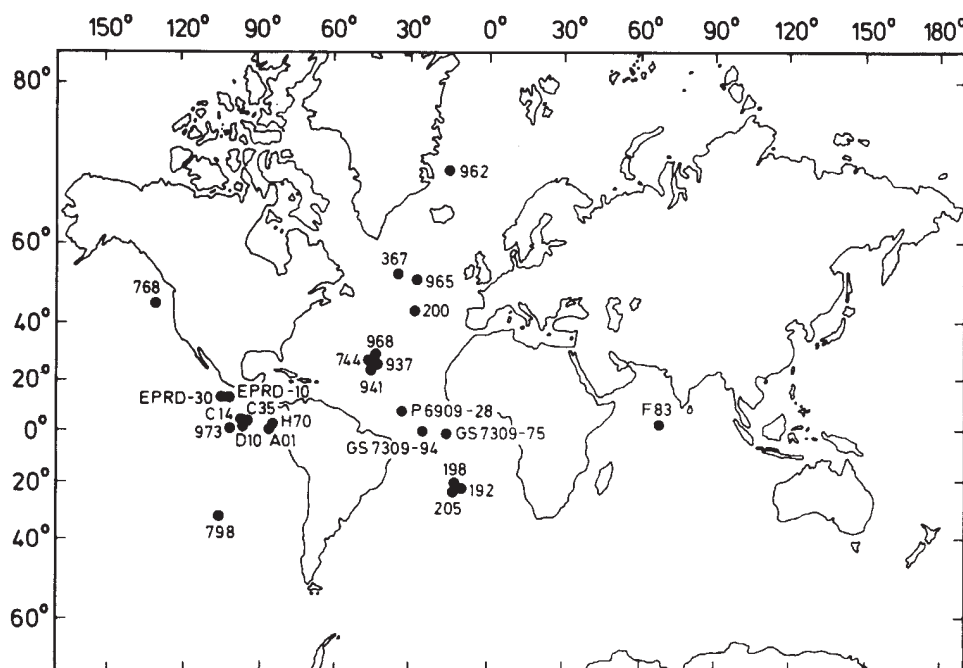
Samples spiked with ⁴⁰K were digested with HF-HClO₄ and separated, along with Rb and Cs, on ion exchange columns containing 4.5 ml of AG50W-X12 ion exchange resin in a bed of 9.5 cm length. The purified alkali fraction was loaded with

Table 1 Locations and analytical results for fresh glasses from dredged MORB samples

Sample no.	Lat.	Location	Long.	K (p.p.m.)	Th (p.p.m.)	U (p.p.m.)	K/U (×10 ⁴)	Th/U
Normal MORB (N-type)								
Atlantic Ocean								
205	22.92° S		13.51° W	793*	0.115	0.0561	1.41	2.05
192	21.93° S		11.81° W	741	0.129	0.0598	1.24	2.16
198	21.87° S		11.85° W	731	0.116	0.0585	1.25	1.98
GS 7309-75	0.55° S		16.07° W	1,600	0.399	0.129	1.24	3.09
GS 7309-94	0.02° S		24.58° W	1,157	0.194	0.0836	1.38	2.32
P 6909-28	6.01° N		33.28° W	1,110*	0.287	0.0993	1.12	2.89
941	22.17° N		45.25° W	1,154	0.234	0.0952	1.21	2.46
937	22.24° N		45.02° W	889*	0.151	0.0662	1.34	2.28
744	25.40° N		45.30° W	1,151	0.206	0.0838	1.37	2.46
968	28.90° N		43.32° W	324	0.0384	0.0255	1.27	1.51
965	49.81° N		28.65° W	632	0.0916	0.0523	1.21	1.75
367	52.67° N		34.94° W	611	0.0995	0.0473	1.29	2.10
962	70.17° N		15.26° W	377*	0.0582	0.0330	1.14	1.76
Pacific Ocean								
798	31.00° S		113.12° W	1,038	0.172	0.0741	1.40	2.32
973	1.45° S		101.35° W	523	0.0856	0.0435	1.20	1.97
D10-1	2.44° N		95.62° W	1,439†	0.306	0.112	1.28	2.73
D10-2	2.44° N		95.62° W	1,597	0.349	0.125	1.28	2.79
D10-3	2.44° N		95.62° W	1,460*	0.316	0.116	1.26	2.72
EPR D-10	12.14° N		103.75° W	1,312*	0.289	0.104	1.26	2.78
EPR D-30	13.83° N		104.18° W	1,558	0.325	0.115	1.35	2.83
768	46.39° N		129.97° W	259	0.0300	0.0215	1.20	1.40
Indian Ocean								
F83	5.35° N		68.68° E	543	0.0846	0.0455	1.19	1.86
Anomalous MORB (P-type and differentiated)								
Atlantic Ocean								
200	42.96° N		29.20° W	4,449	1.59	0.428	1.04	3.71
Pacific Ocean								
A01	0.71° N		85.50° W	1,437	0.726	0.256	0.561	2.84
H70	1.04° N		85.12° W	3,116	1.09	0.276	1.13	3.95
C14	2.70° N		95.24° W	9,173†	5.42	1.79	0.512	3.03
C35	2.70° N		95.24° W	4,370†	2.09	0.583	0.750	3.58

K analyses: * ID-SSMS; † thermal ionization MS; or otherwise average of both methods.

Fig. 1 Sample locations of fresh glasses from dredged MORB.



phosphoric acid on single Ta filaments and isotope ratios measured using a Finnigan-MAT 261 mass spectrometer. The use of the ^{40}K spike allowed for fractionation correction. Three replicate analyses of US Geological Survey (USGS) standard BCR-1 yielded a mean of 1.419% K and a standard deviation of 1.1%.

Results

The concentrations of K, Th and U and the ratios of K/U and Th/U are listed in Table 1. The concentrations vary by more than an order of magnitude between samples, but the ratios of K/U and Th/U are much more uniform (Figs 3, 4). In this respect, these elements resemble the highly incompatible elements Ba, Rb and Cs (ref. 26) much more closely than the heavy REE, Hf, Zr and Sr, all of which have comparatively uniform concentrations in MORB. This behaviour may be the consequence of variable degrees of source depletion and/or variable degrees of partial melting. Additional variations in the trace element concentrations are imposed by variable degrees of crystal fractionation, which is particularly prominent in the samples A01, C35 and C14 (Fig. 2).

K/U

Figure 3 shows approximately constant K/U ratios for the depleted MORB. There is no evidence for differences in the ratio of these elements in the various parts of the ridge system. The uniformity of K/U is remarkable considering, for example, the well-known variation of the K/Rb ratio, which ranges from about 400 to 2,000 (ref. 26). K and U are not as highly incompatible as Th (see below) or as Rb, Ba, Cs (ref. 26). They have different ionic charges and radii, and there is no obvious geochemical affinity between them. Nevertheless, K and U must have nearly identical effective bulk partition coefficients in the petrogenetic processes (source depletion, partial melting) relevant to the genesis of MORB. This may be the result of a fortuitous balance between the effects of radius and charge. A similar effect has been observed for Ti and Sm (ref. 27).

The significantly lower K/U ratios encountered in the fractionated basalts A01, C14, C35 may be a result of plagioclase crystallization. It has been shown by Morse²⁸ that plagioclase in crystallizing magma chambers can have a rather high partition coefficient for potassium, and this effect might explain the anomalously low K/U ratios in fractionated MORB.

Our result of $\text{K/U} = 1.27 \times 10^4$ in depleted MORB strongly supports the postulated bulk-Earth value of 10^4 of Wasserburg

*et al.*¹ who based their estimate on the abundances in crustal rocks. This ratio is now much more closely constrained than was indicated by the data reviewed by O'Nions *et al.*¹², some of which may have been affected by seawater alteration. Furthermore, our results are not in agreement with the lower estimates of K/U made by Fisher²⁹, Stacey³⁰ and Sleep¹³ on the basis of ^4He and ^{40}Ar abundances.

Th/U

The Th/U ratio varies smoothly from 1.4 to 3.1 as Th increases from 0.030 to 0.40 p.p.m. All samples from the different parts of the ridge system lie on the same curve (Fig. 4). The results of Condomines *et al.*³¹ for FAMOUS basalts are in good agreement with these data but lie mostly on the horizontal part of the curve, that is at relatively high Th concentrations. The data of Sun *et al.*³² for basalts along the Reykjanes ridge cover a wide range of Th concentrations, and their Th/U ratios follow a similar trend but show a larger scatter.

The Th/U ratios reported here contain some of the lowest values found so far in any oceanic basalts. It might be suspected that some of these low values are the result of U uptake from seawater. We consider this to be unlikely for the following reasons: (1) the samples were selected and hand picked with the utmost care to exclude any kind of altered glass; (2) the plotted Th/U versus Th values show an extremely low degree of scatter; (3) Sr and O isotopic compositions and Ba-Rb-Cs data (to be published elsewhere) show no evidence of alteration.

Primitive mantle K, K/U and K/Rb

The terrestrial potassium concentration and K/Rb ratio have commanded the attention of geochemists^{3,4,33-37}. Our results, combined with the evaluation of the terrestrial Ba-Rb-Cs relationship²⁶, provide new estimates on the K abundance, the K/U and the K/Rb ratio of the silicate portion of the Earth (crust plus mantle, also called 'primitive' mantle).

An assessment of the crustal K/U ratio is made difficult by the well-known depletion of U in the lower crust and is beyond the scope of this paper. However, if we accept the value of 1×10^4 of Wasserburg *et al.*¹ as representative of the crust, we find that the crustal value is indistinguishable from the depleted-mantle value. Furthermore, if we accept that the continental crust and the depleted mantle are geochemically complementary, that is, that the primitive mantle was differentiated into continental crust and depleted mantle^{2,12,38}, it follows that the K/U ratio (being the same in both complements) was not

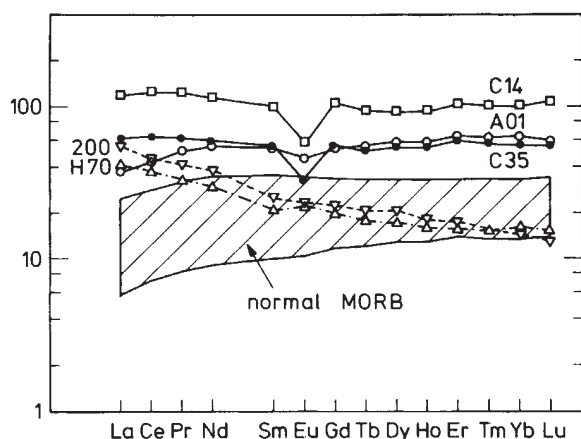


Fig. 2 Concentrations of the REE normalized to C1-chondrites. Most of the investigated samples belong to the normal type (N-type MORB). Anomalous MORB are the samples 200 (Mid-Atlantic Ridge, 43°N) and H70, A01, C35, C14 (Galapagos Rise). A01, C35, C14 are differentiated with FeO/MgO ratios of 3.74, 2.61, 12.42 (ref. 22) respectively.

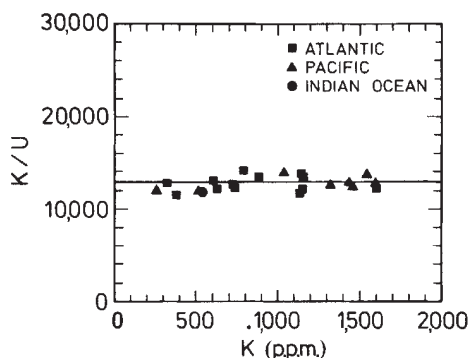


Fig. 3 K/U versus K in all N-type MORB. The horizontal line represents the arithmetic mean of $K/U = 12,700 \pm 200$ s.e.m.

affected by the differentiation and the value $K/U = 1.27 \times 10^4$ is also representative of the primitive mantle and therefore the entire silicate portion of the Earth. Because of its refractory nature, the U abundance in the primitive mantle is known to rather narrow limits. Primitive-mantle abundances of such elements are about 2.6 times their value in C1 chondrites or double the value in ordinary chondrites^{7,36,37,39,40}. Using 0.008 p.p.m. U for C1^{14,41}, we obtain a primitive-mantle abundance of $U = 0.021$ p.p.m. and $K = 12,700 \times 0.021$ p.p.m. = 267 p.p.m.

The K/Rb ratios can be derived from the terrestrial K/U and Ba/Rb ratios combined with the meteoritic U/Ba ratio, assumed to be identical to the terrestrial ratio because both U and Ba are refractory and lithophile elements. We use a terrestrial Ba/Rb = 11.3 derived from both enriched and depleted oceanic basalts²⁶. This value is also in fairly good agreement with the upper crustal estimate of Ba/Rb = 9.7 by Shaw *et al.*⁴². Ba and U data for chondritic meteorites scatter and we are not aware of published analyses of both U and Ba on the same meteorite aliquots. Averages for seven chondrite classes compiled by Mason⁴³ give $Ba/U = 282 \pm 45$. The data compiled by Anders and Ebihara⁴¹ give $Ba/U = 280$ for Orgueil. Using the latter value, we obtain a terrestrial $(K/Rb) = (K/U) \times (Ba/Rb) \times (U/Ba) = 513$, a value that is significantly higher than the Orgueil $K/Rb = 250$ (ref. 41). In this context, we mention the terrestrial $Rb/Cs = 80$ inferred by Hofmann and White²⁶ on the basis of arguments similar to those used for Ba/Rb. This value shows an even greater difference from the C1-chondritic $Rb/Cs = 12$. These results imply a progressive

alkali depletion ($K < Rb < Cs$) of the silicate portion of the Earth relative to the primitive chondrites. These Rb/Cs, K/Rb and K/U ratios can be further tested when better estimates of these ratios in the bulk continental crust are available.

Depletion mechanism

The samples analysed for this study belong to the class of ordinary MORB, which are known to be derived from mantle regions that are depleted in incompatible elements. The evidence for this depletion is firmly established and need not be reviewed here. One manifestation of this source depletion is the higher-than-primitive K/Rb ratios in depleted MORB²⁶. The Th/U ratios reported here are another such manifestation. These ratios range from about 1.4 to 3.1 and are all distinctly lower than the value of the primitive mantle, $Th/U = 3.6-4.0$. The positive correlation of Th/U versus U (Fig. 4) shows that Th is more highly incompatible than U, because the three major igneous mechanisms, variable partial melting, variable fractional crystallization, and variable source depletion, all result in such positive correlations (although with drastically different slopes), if the more highly incompatible element is placed in the numerator. The more highly incompatible nature of Th (compared with U) may also be inferred from its slightly larger ionic radius. Given this order of incompatibility and the lower-than-primitive Th concentration in the most depleted samples, the lower-than-primitive Th/U ratios found in all samples are best explained by the depletion process itself. Partial melting and crystal fractionation processes would increase Th/U. U recycling through subduction of altered oceanic crust may contribute to the lowering of Th/U in the mantle⁴⁴, but the evidence for recycling is found in the enriched oceanic island basalts, not MORB. If the depleted mantle regions contain any recycled material, its contribution must be small.

Depletion model

Figure 5a shows the chemographic relationships in depleted residues compared with those in equilibrium partial melts. Both processes produce approximately straight lines on the diagram chosen (see ref. 45 for a discussion of partial melting; the analogous case of depletion processes will be treated elsewhere). Neither mechanism alone fits the observed Th-U relation. We therefore propose the following depletion mechanism: a fluid phase, such as a melt, is formed and partially removed from the primitive source. This fluid has the element ratios $K/U = 1.27 \times 10^4$ and $Th/U \sim 3.3$. The solid residue has the same K/U ratio and $Th/U \sim 1.0$. The total residue consists of a mixture of residual solid and fluid (melt) left behind. Different residues with different proportions of residual solid and melt define a mixing hyperbola that characterizes the model source. This hyperbola is modified in the final MORB because of the effect of partial melting. In Fig. 5b the degrees of partial melting and subsequent fractionation are assumed to be constant for all samples, in order to illustrate the simplest case. The important point is that the effects of depletion and remelting cannot be completely random, otherwise the data would not conform to the observed systematic pattern.

An important semiquantitative result of our depletion model, the details of which will be published elsewhere, is the small degree of melting required by all successful combinations of partition coefficients and fluid fractions withdrawn during depletion. Only in this way is it possible to match the observed ratio changes with the observed concentrations. The physics of extracting such small fractions of a fluid phase is an important unsolved problem, which is beyond the scope of this article.

Heat production

We cannot translate the concentrations measured in MORB directly into source concentrations (and therefore into heat production), because the enrichment factor caused by partial melting and possible subsequent fractional crystallization is not well known. We can, however, derive an estimate of the

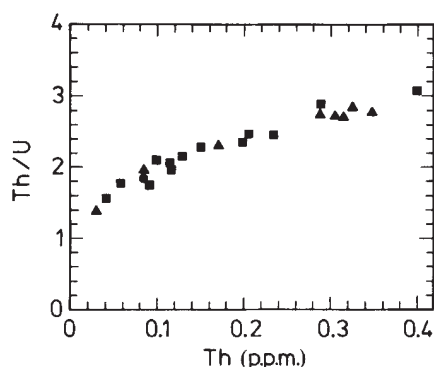


Fig. 4 Th/U versus Th in all N-type MORB. Symbols as in Fig. 3.

maximum possible concentrations of Th, U and K in the source mantle, using only the ratios of U (or Th or K) to less depleted refractory elements such as Zr, Hf, Y and the heavy rare earth elements (HREE).

We make the following assumptions: (1) The abundances of the refractory elements, U, Th, Zr, Hf, Y, REE, in the Earth's primitive mantle are known^{16,36,37,40}. (2) These elements are all depleted, to an unknown degree, in the present-day depleted mantle. (3) U, Th and K are at least as highly depleted as the moderately incompatible elements Zr, Hf, Y, HREE. We have chosen these particular elements, because they have the highest enrichment factors in MORB relative to primitive mantle. Our own (unpublished) data on the same set of samples (see also Fig. 2) and a wealth of published data on MORB (see ref. 46) show that the average enrichment factor (over primitive mantle) is approximately equal to 10. Choosing Zr as an example, we have the following relationships for the concentration of Zr and the U/Zr ratio in the MORB mantle (MORBMA) and in the primitive mantle (PRIMA):

$$Zr_{\text{MORBMA}} \leq Zr_{\text{PRIMA}}$$

and

$$(U/Zr)_{\text{MORBMA}} \leq (U/Zr)_{\text{MORB}}$$

It follows that

$$U_{\text{MORBMA}} = (U/Zr)_{\text{MORBMA}} \times Zr_{\text{MORBMA}} \\ \leq (U/Zr)_{\text{MORB}} \times Zr_{\text{PRIMA}}$$

Using values of Zr = 10 p.p.m. in the primitive mantle, Zr = 100 p.p.m. and U = 0.08 p.p.m. in average depleted MORB, we find a maximum value of U in the source mantle for depleted MORB of U = 0.008 p.p.m.

An alternative method of estimating the concentrations of highly incompatible elements in the depleted mantle is based on isotopic abundances of Sr and Nd. Jacobsen and Wasserburg² calculated Rb concentrations for two mantle evolution models, one in which an increasing mass fraction of the mantle is subjected to a constant degree of depletion, and one in which a constant mass fraction of the mantle is depleted progressively. Combining these evolution models with a simple equilibrium partial melting model, they computed two internally consistent sets of model parameters. The Rb concentrations obtained in this way for the depleted mantle reservoirs are 0.044 and 0.041 p.p.m. respectively. Using a rounded value of Rb = 0.04 p.p.m. and an average K/Rb = 1,000 (see ref. 20), we obtain a K concentration of ~40 p.p.m. From our K/U = 1.27×10^4 , and Th/U ~ 2, we find U ~ 0.003 p.p.m. and Th ~ 0.006 p.p.m. These concentrations yield a total present-day heat production of 0.6 pW kg⁻¹. The corresponding maximum values (obtained in our calculation given above) are U = 0.008 p.p.m., Th = 0.016 p.p.m., K = 100 p.p.m. and a heat production of 1.5 pW kg⁻¹. The concentrations in the primitive mantle are U = 0.021 p.p.m., Th = 0.075 p.p.m. and K = 267 p.p.m., and the heat production of the primitive mantle is 5.0 pW kg⁻¹. O'Nions *et al.*¹² originally based

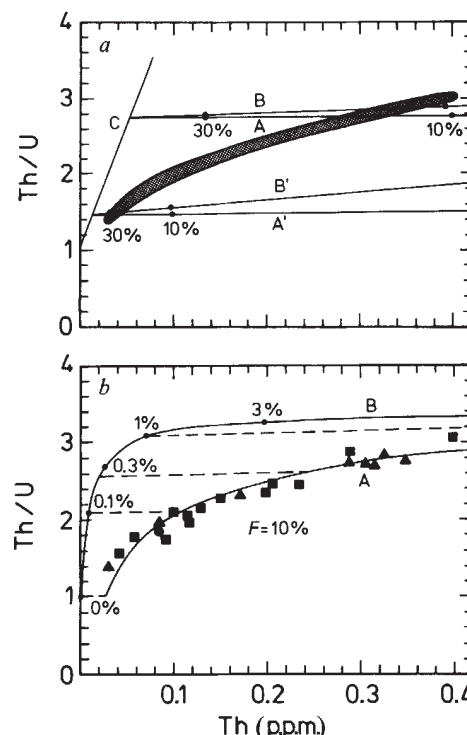


Fig. 5 *a*, Chemographic relationship of Th/U versus Th in depleted residues (C) compared with those in equilibrium partial melts (A, A'; B, B'). The following bulk partition coefficients are assumed: $D^U = 0.001$, $D^{Th} = 0.0003$ (A, A') and $D^U = 0.01$, $D^{Th} = 0.003$ (B, B'). For the partial-melt lines, the values for degrees of partial melting of 10% and 30%, respectively, are plotted. The locus C is the solid residue of a melt extraction from primitive mantle (Th/U = 3.6, Th = 0.075 p.p.m.). It is nearly identical for the two sets of partition coefficients. The shaded area represents the analytical data shown in Fig. 4. *b*, Hypothetical chemographic relationship of partial melts (curve A) formed from depleted mantle (curve B). Curve B is the locus of mixtures between a depleted crystalline residue (Th/U = 1, U = 0.002 p.p.m.) and a residual melt or fluid (Th/U = 3.3, U = 2 p.p.m.) left behind after melt extraction of an approximately primitive mantle. The percentage values given on curve B correspond to the proportions of melt in the mixture. The value of $F = 10\%$ is the degree of remelting this mixed source (B) to form MORB. F is assumed to be constant. The quantitative model described is not unique and is intended as an example only.

their estimate of heat production in the primitive mantle (13 pW kg⁻¹) and in the depleted mantle (4.5 pW kg⁻¹) on the assumption that terrestrial heat production and heat loss are balanced. Subsequently, they published estimates based on chemical data (as we have done here) and obtained values of 4.8 pW kg⁻¹ for the primitive mantle and 1.2 pW kg⁻¹ for the depleted mantle⁴⁷. The important point of agreement is that heat production in the depleted mantle is <30% and probably as little as about 10% of the heat production in the primitive mantle. Therefore we confirm the conclusion¹² that most of the heat lost through seafloor spreading is produced in mantle reservoirs external to the MORB-source mantle. This result should serve as a useful constraint for models of the thermal history of the Earth and the study of mantle convection.

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A reinterpretation of mammalian sodium channel gating based on single channel recording

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Some of the traditionally held views about how sodium channels work are shown to be incorrect and a new approach to physical theories of sodium channel operation is suggested.

OUR notions about the molecular basis for the nerve impulse derive primarily from experimental and theoretical results of Hodgkin and Huxley¹ on the squid giant axon. Their theory is now interpreted as describing the behaviour of a voltage-regulated integral membrane protein, known as the sodium channel, that plays the central role in generation of nerve impulses. Other workers have found that the same general description, with a number of important modifications, is satisfactory for sodium channels of various other preparations. We report here experiments suggesting that, although the Hodgkin-Huxley description is formally adequate, a new interpretation in terms of molecular behaviour is required for at least some mammalian sodium channels.

When the voltage across a resting excitable membrane is changed stepwise to a value sufficiently more positive than the resting level, the permeability of the membrane to sodium rapidly increases and then more slowly declines despite the fact that the membrane voltage is maintained at the positive value that induced channel openings. This increase and subsequent decrease in sodium permeability implies that the sodium channels can progress through at least three distinct functional states: resting, activated and inactivated. Thus, a sufficiently positive voltage causes sodium channels to make the transitions from a (closed) resting state to an (open) conducting state and a (closed) inactivated state, distinct from the resting state. Hodgkin and Huxley identified the rapid increase in permeability with the activation process (progression of channels from resting to activated states) and the subsequent decline with inactivation (entry of channels into the inactivated state). One of their fundamental assumptions was that the two processes are kinetically distinct: activation is rapid and inactivation is slow. Subsequent workers have produced experimental support for this assumption in some preparations by discovering pharmacological agents that appear to remove inactivation while leaving the activation mechanisms unaltered^{2–9}.

Our recent studies have shown this assumption, made originally by Hodgkin and Huxley, to be incorrect for at least

some mammalian sodium channels. Specifically, we find that although these mammalian cells exhibit a rapid rise and slow decline in sodium permeability much like in the squid, their sodium channel activation process can be slow and rate limiting whereas inactivation is uniformly rapid. As we describe below, the rate of decline in sodium permeability is not, for these mammalian cells, a manifestation of inactivation time course. Analysis of the molecular mechanisms of voltage dependent gating of course presupposes that these separate processes are correctly identified and characterized.

The response of sodium channels to a step change in voltage is shown in Fig. 1: immediately after the transmembrane voltage is changed from -100 to -20 mV (relative to the cell's resting potential), the sodium permeability (measured here by the probability of a sodium channel being open) rapidly increases and then more slowly decreases. The declining phase has been fitted here by an exponential with a time constant $\tau_h = 1.9$ ms and the mean open time for individual channels, as will be described below, was 0.5 ms. A traditional analysis would assign the decay time constant $\tau_h = 1.9$ ms to the inactivation process, and would suppose that activation was complete soon after the peak permeability. Our analysis, presented here, shows that τ_h represents a slow component of activation, and that inactivation occurs more rapidly, essentially with a time constant equal to the mean open time of 0.5 ms.

Experimental methods

We have used the neuroblastoma cell line N1E115 for most of our studies, but have in some instances investigated primary culture of rat myotubes and a pituitary cell line GH₄C₁. Single-channel recordings were made using the method originally described by Sigworth and Neher¹⁰ (see also Hamill *et al.*¹¹). Most experiments used cell attached patches, but we have also studied channels in the inside-out and outside-out configurations on some occasions. We have found that cell attached patches tend to remain stable for much longer times. Because we used cell attached patches, all voltages specified here are relative to

the resting potential of the cell. Our observations are based on studies of channels in something over 50 patches. Each patch contained from one to approximately 12 sodium channels. We have noticed a tendency for even numbers of channels to occur in a patch; for example, only one patch has had a single channel, whereas two and four channel patches are fairly common and three channel patches are relatively rare.

The measurements we have made are illustrated in Fig. 1. At the top of the figure are seven specimen records of single channel currents. For each record we have determined: (1) the first latency, that is, the time from the onset of depolarization to the first channel opening, (2) the dwell time in the open state for each opening, and (3) the number of channel openings in each trace. In addition, we have averaged groups of individual records together to find the probability of a channel being open as a function of time; a typical result of this averaging process is shown in the lower part of Fig. 1.

Sodium channel behaviour

We have confirmed the observation that neuroblastoma sodium channels¹²⁻¹⁴ behave in a manner typical of other preparations. Figure 2A shows a family of averaged currents obtained for a variety of voltages. Approximately 64 traces were averaged for each voltage displayed. The average currents displayed in Fig. 2A are similar to macroscopic currents studied in other preparations.

Figure 2B shows the conductance at the peak of the current transient normalized to the maximum value and plotted versus voltage. This activation curve has the familiar S-shape. Time constants for the decaying phase τ_h are plotted versus voltage in Fig. 2C and show the form of voltage dependence usually found for this parameter. The data in Fig. 2 are characteristic of sodium channel behaviour in all preparations.

Activation and inactivation

Our goal has been to investigate the inactivation and activation processes separately. To study activation, we have determined the first latency distribution function. This function specifies the probability that an individual channel will open for the first time after t ms have passed since the onset of a depolarizing step change in voltage. The first latency distribution function describes the probability of first passage time from a starting state into the open state and reflects rate constants between various non-open states including the rate of inactivation of closed channels.

Our investigation of the inactivation rate for open channels begins with a characterization of duration distribution functions (the probability that a channel remains open for t or more ms). We then consider the question: what fraction of open channels close directly into the inactivated state rather than revisiting a state along the pathway to opening? The rate of open channel inactivation can then be determined, as we shall describe, from mean open duration and the fraction of open channels that close directly into the inactivated state.

In the following discussion we will focus first on the properties of the first latency distribution function and then return to the questions about inactivation and its characterization.

Latency function

In a patch containing only a single sodium channel, determination of the first latency distribution function is quite straightforward: the patch is depolarized for 15 ms once every second, and the time between the onset of the depolarization and the first channel opening is recorded with an accuracy determined by the sample rate, usually 0.1 ms in these experiments. A cumulative histogram is then constructed which specifies the relative frequency with which first openings occurred later than each sample point.

For a patch containing more than a single channel the determination of the true first latency distribution function is complicated by the fact that the time until first opening depends on not only activation of individual channels but also the number

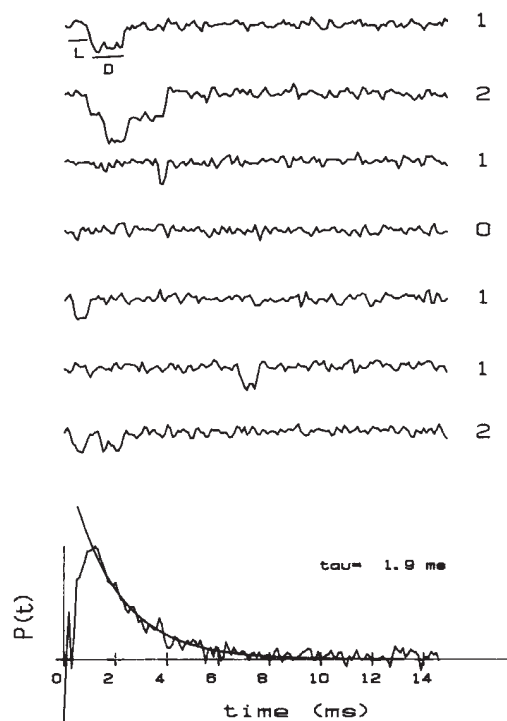


Fig. 1 Inward currents through single sodium channels. Repetitive depolarizing steps from a holding potential of -100 to -20 mV relative to the resting potential of the cell were applied to a cell attached patch at an interval of 0.8 s. Four sodium channels were present in the patch. Seven representative records are shown to illustrate the variables that were analysed. Leakage and capacitive currents were digitally subtracted. The single channel current is ~ 1.2 pA. Latency until first opening and open channel durations were measured by computer. These are indicated by the letters L and D beneath the first record. The number of openings is indicated to the right of each record. Beneath these traces is the channel open probability transient calculated by averaging several hundred records (including the seven illustrated). The falling phase of this transient has been fitted with a single exponential called τ_h . It is similar to τ_h measurements from macroscopic sodium currents. Durations of opening events that overlap in time were measured by randomly assigning pairs of the opening and closing transitions. For example, when two openings overlapped, the first closing event was paired with the first opening event half of the time and with the second opening event half of the time. The error introduced by this method is small when the patch contains a small number of channels. The currents were filtered at 2 kHz, the temperature was 9.5°C .

of channels present. If the channels are identical and independent, however, the true first latency distribution can be obtained from the apparent one by simply taking the N th root of the apparent distribution function where N is the number of channels present in the patch. The number of channels present is estimated simply by inspecting between 100 and 2,000 records and counting the maximum number of channels open simultaneously; this procedure gives a lower limit on the actual number but is accurate if the number of channels in the patch is no more than about 4 or 5, a sufficient number of large depolarization steps are used, and the holding potential is negative enough so that there is little resting inactivation. We have found this simple counting method seems to work as well as more complicated statistical determinations of channel number.

First latency distributions shown for three different potentials in Fig. 3 contain at least two distinct rate constants (reciprocal time constants) and can be approximately fitted by the function

$$F(t) = (1 - P_0)(R_1 e^{-R_2 t} - R_2 e^{-R_1 t}) / (R_1 - R_2) \quad (1)$$

The rapid and slow rate constants, R_1 and R_2 , present in the first latency distribution both get larger with increasing

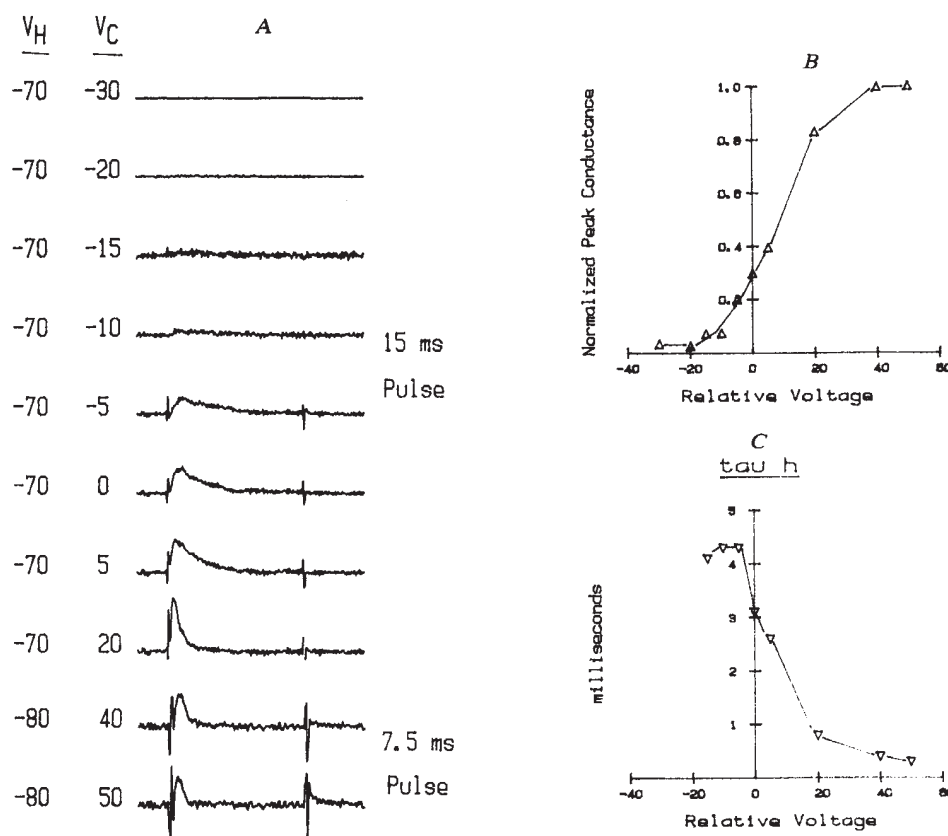


Fig. 2 Voltage dependence of sodium channel kinetics. **A** shows average currents for steps to various command voltages. Voltages are relative to rest potential. The currents can be seen to increase in speed as the membrane is more depolarized. **B** shows the peak conductance for each of these averages normalized to the maximum and plotted against voltage. **C** is a plot of τ_h versus voltage. The patch contains at least 9 channels. Filtered at 2.5 kHz. Temperature: 20 °C.

magnitude of depolarization. Note that the asymptotic value P_0 of the first latency distribution is not zero; this non-zero asymptote reflects the fraction of channels that pass directly into the inactivated state without ever opening (see ref. 15).

The most important feature of the first latency distribution function is that the smaller rate constant is comparable to the rate for the declining phase of the sodium current, at least over most of the activation range shown in Fig. 2. Many channels are opening for the first time during the process that normally would be described as inactivation. Thus, the activation process takes place throughout the sodium current transient and plays an important role not only in the initial rise to a peak but also in the decline: activation has very slow components.

Rate of open channel inactivation

A channel, once open, remains open for a random length of time. The duration distribution function $L(t)$ that describes this behaviour is obtained from single channel current records by measuring the duration for each channel opening and compiling

a histogram that specifies the relative frequency of finding an open time of t ms or longer. The durations are approximately exponentially distributed and show the interesting feature that, over most of the activation voltage range (see Fig. 2), the mean open times (and in fact the entire distribution function) are independent of membrane potential. Mean open times at room temperature are close to 0.5 ms. Fenwick *et al.*¹⁶ have reported a decrease in mean open duration at low depolarizations where there is little activation. We have also found shorter durations at depolarizations where the probability of a channel opening is low (−30 to −15 mV in Fig. 2). It is clear, however, that over all but the lowest part of the activation range the duration is independent of membrane potential.

The dwell time in any state is the reciprocal of the sum of the rates for leaving that state. For an open channel, then, the open duration reflects the rate of returning to a state visited prior to the opening and also the rate of inactivation. Specifically, if a is the rate of revisiting the opening pathway and b is the rate of progressing to an inactivated state (or states), the mean

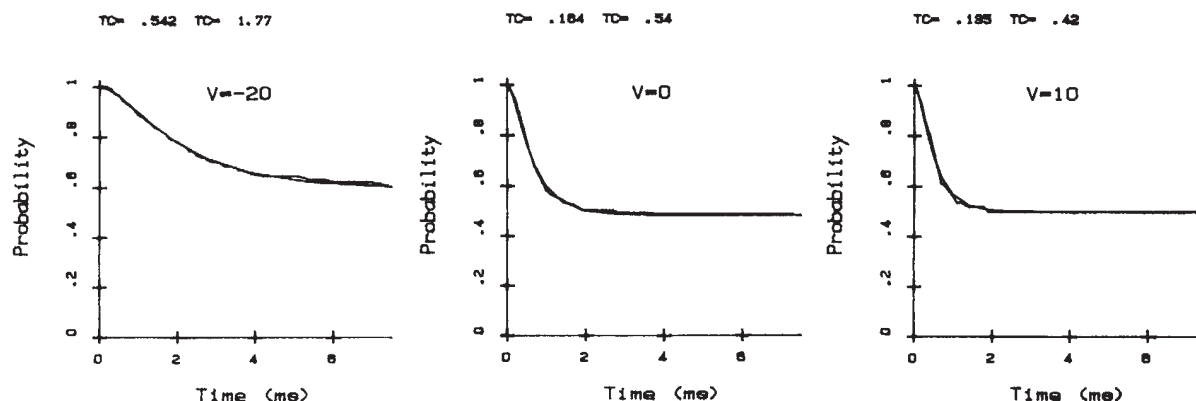
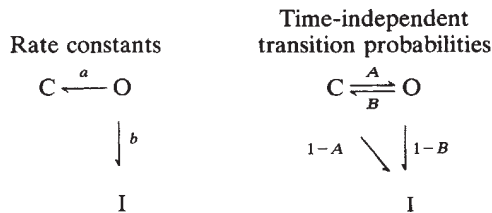


Fig. 3 First latency functions at three voltages. These are calculated as described in the text. Superimposed on each is a fit to equation (1) with time constants (reciprocals of the rate constants R_1 and R_2) indicated above the curves (in ms). As the membrane becomes more depolarized both of the time constants in the fits become faster. This patch contained four channels. Temperature, 16 °C.

open time is $1/(a+b)$; furthermore, the fraction of closings from the open state into the inactivated state is $b/(a+b)$. If we can estimate the fraction of transitions that proceed to the inactivated state, then the rate constant for inactivation can be calculated from this fraction and the mean open time.

We consider three groups of states: resting, open, and inactivated. The channel starts in one of the resting states, we observe entrance into the open state, and we know that channels rarely if at all return from the inactivated state at the membrane voltages that produce openings; the inactivated state is an absorbing one. Consider now transitions between these states (or groups of states) without regarding time; that is, we ask only for the probability of a transition to a particular state, whenever it occurs.



For example, at a particular voltage, the probability of the transition from closed (C) to open (O) may be A , which would mean that the probability of entering the inactivated state (I) from resting states would be $1-A$. $1-A$ can be estimated from the number of records in which a depolarizing voltage is maintained for a sufficiently long period of time and no channel opening occurs. Similarly, a channel in the open state may pass directly to the inactivated state with a probability of $1-B$ or it may return to one of the resting states, when the transition occurs, with the probability B . If we can estimate A and B , we can then calculate the rate of inactivation from the mean open time and the relation $1-B = b/(a+b)$.

The situation just described is a Markov chain with an absorbing state (inactivated). Straightforward calculations reveal that, for a single channel, the probability of observing k transitions into the open state before the channel enters the absorbing (inactivated) state $P(k)$ is given by

$$P(k) = P_0(O) \left(\frac{1-AB}{B} \right) (AB)^k \quad (2)$$

where $P_0(O)$ is the probability of being in state C initially; this probability specifies the resting inactivation. For an N -channel patch, the probability of observing k openings per depolarizing step is given by the N -fold convolution (denoted by N^*) of the distribution (equation (2)):

$$P(k)_N = \{P(k)\}^N \quad (3)$$

We have used these relations to estimate the probability $1-B$ that an open channel will pass directly into the inactivated state by observing the relative frequency of k transitions into the open state for patches with various numbers of channels from 1 to 4. $1-A$ has been estimated by the N th root of the fraction of records with no opening and then $1-B$ found by fitting the data by equation (3). A histogram giving the relative frequency of k transitions per trace for a 4-channel patch is shown in Fig. 4; data in the figure have been fitted with equation (3), and $1-B = 0.79$. In all cases, the probability that a channel makes a transition directly from open to inactivated is between about 0.8 and 1, and the fraction of open channels entering the inactivated state appears not to depend on membrane potential (see Fig. 5). In other words, channels usually close by inactivating at all voltages throughout the activation range (see Fig. 2).

The calculation described above makes several idealizing assumptions which are difficult or sometimes impossible to check directly. For example, we obtain a lower limit for the number of channels in the patch by observing the number simultaneously open, but we cannot be sure the patch does not contain more channels than we suppose. Furthermore, we have assumed that the inactivating state is absorbing, whereas an inactivated chan-

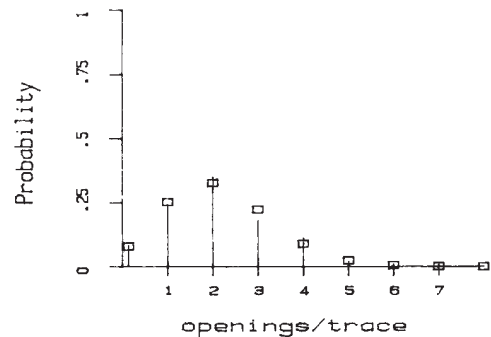


Fig. 4 Histogram of the number of openings per depolarizing step to -20 mV from a holding voltage of -90 mV (relative to the resting potential). Superimposed on the histogram is the solution of equation (3). The probability of a channel opening $A = 0.47$ and the fraction of openings which closed to the inactivated state $1-B = 0.79$.

nel may occasionally reopen. Also we have neglected the fact that there must be several different resting states and have instead assumed there to be only one. Each of these assumptions, if incorrect, would give an underestimate for the fraction of channels closing directly into the inactivated state. For these reasons the fraction of channels closing into the inactivated state given above is a lower limit of the actual value, and channels must close into the inactivated state at least 80 or 90% of the time.

Since the open channels predominate close to the inactivated state and do not reopen, the mean open time is essentially the reciprocal of the inactivation rate constant. Since the durations are much shorter than τ_h at most voltages (see Fig. 5), the time course of the average sodium current must be predominately determined by activation. At any time during the transient, current is flowing through channels that only recently opened for the first time.

An alternative approach

We have concluded, then, that activation is the rate limiting step, and that inactivation is rapid and is not significantly dependent on voltage over the voltage range where activation occurs. These conclusions have been reached by examining the distribution of times until first opening, the distribution of open durations, and the probability that an open channel enters the inactivated state when it makes the closing transition. Our conclusion can be checked in another way as indicated by the following argument. The probability that a channel is open at time t is given by the equation

$$p(t) = \int_0^t f(t-\tau) M(\tau) d\tau \quad (4)$$

where $p(t)$ is the probability of finding a channel open at time t , $f(t)$ is the probability density for a channel first opening at time t and $M(t)$ is the probability of finding a channel open at time t given that it first opened at time 0. This convolution integral enumerates the ways a channel can open at all times previous to t and then be found open when observed at time t . The function $M(t)$ can be further decomposed into the probability that a channel has not closed by t and the probability that a channel closed before t and then was found open when observed at time t . Insofar as channels usually close directly into the inactivated state, reopenings are negligible and the function $M(t)$ is just the duration distribution function $L(t)$ that we have determined experimentally. Because both the functions f and L can be measured directly from our data, the convolution of these two functions should reproduce the observed probability of a channel being open if each channel only opens one time. We have tested this equation and found it to be accurate.

Figure 6 shows the convolution of duration and latency distributions superimposed on average currents at three voltages.

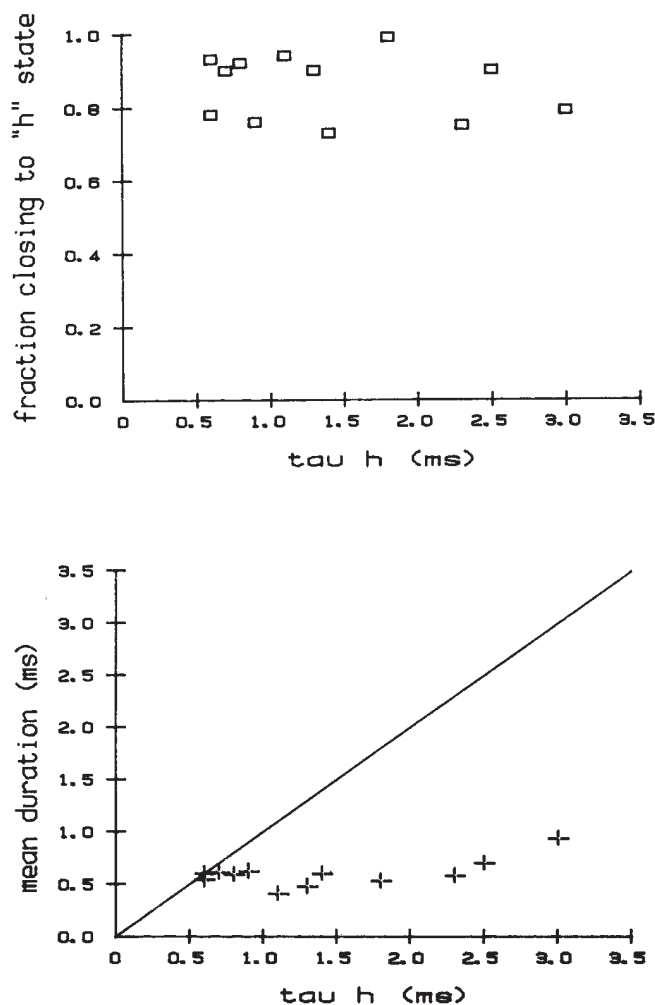


Fig. 5 The fraction of channels closing to the inactivated state and the mean duration are plotted versus values of τ_h from the corresponding average. Data are pooled from many different patches, for various depolarizations. τ_h was used for the abscissa instead of voltage to permit comparison of patches from different cells which may have had different resting potentials. τ_h can be approximately translated into voltage by using the τ_h versus V curve in Fig. 2. The fraction closing to the inactivated state was calculated as detailed in the text and Fig. 4. The straight line shown in the lower panel would be expected if τ_h were determined by the open durations. Temperature ranged from 16 to 21.8 °C.

Agreement between predicted and observed functions is good. If the channels reopened appreciably, the experimental averages would decay more slowly than the calculated ones. Note also that as the average currents get faster with higher depolarizations the duration distributions remain similar but the first latency distributions become faster.

Conclusions

Our results suggest that channels open only once per depolarization epoch; they primarily close to an inactivated state and do not reopen. The inactivation rate is fast and not very voltage dependent. Slow components of activation are rate limiting except at those voltages for which τ_h approaches its asymptotic value (see Fig. 2).

These conclusions are different from the Hodgkin and Huxley interpretation of activation and inactivation. They agree in some ways, however, with more recent work on squid and amphibian axons. In particular, based mainly on the lack of a gating current component with the time course of inactivation, Armstrong and Bezanilla¹⁷ and Nonner¹⁸ have concluded that inactivation is

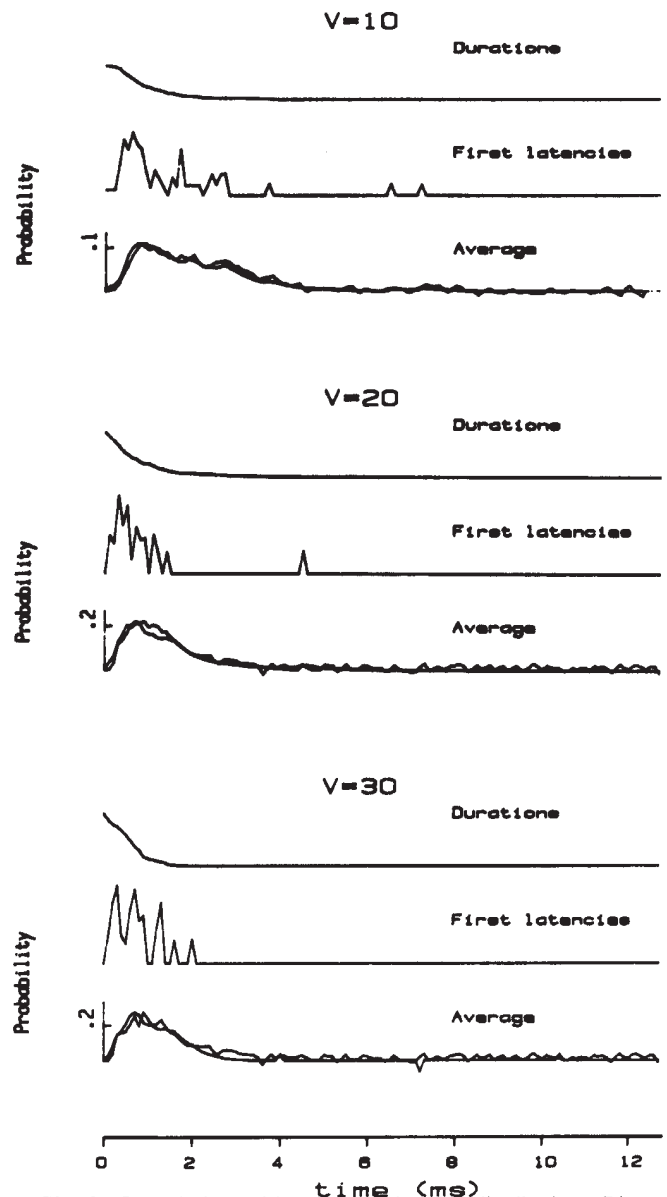


Fig. 6 Convolutions of latency and duration distributions. Displayed for each voltage are: duration distribution functions; first latency probability density functions (calculated by taking the derivative of first latency functions such as in Fig. 3); the convolution of these two functions; and the corresponding probability transient (noisier trace). Convolutions were performed by multiplying the Fourier transforms of the two functions and taking the inverse transform of the product. Individual points plotted in this figure are connected by straight lines. The patch contained three channels. Temperature, 14.5 °C.

not voltage dependent (but see ref. 19). They have shown that, at a number of voltages, the time course of sodium current and gating currents can be well fitted by a model in which inactivation rates are not dependent upon voltage^{4,18,20}. Our results, for single sodium channels from mouse neuroblastoma, are in agreement with their interpretation.

There is a discrepancy, however, between neuroblastoma and squid and amphibian sodium channels in regard to slow components of activation. Experiments in these tissues with agents that remove sodium channel inactivation, such as pronase^{2,3,9} and *N*-bromoacetimide (NBA)^{7,8,21} show that activation is essentially over by the time the current reaches its peak. This is in contrast to our first latency results and to single channel experiments on NBA-treated sodium channels from rat myotubes²². These authors find that after NBA treatment (presumably removing inactivation), activation continues long after

the time of peak current in untreated channels. These differences may reflect a difference between mammalian and other sodium channels. Mammalian sodium currents have been found in some cases to have much faster inactivation than squid or amphibian channels²³. If the inactivation rate is fast, peak current will occur at earlier times than if it is slow and the probability of a channel being open at the peak will be less than if inactivation is slower. Consequently, activation will not be complete by the peak. In tissues where inactivation is slow relative to activation (such as squid axon and amphibian nerve) we would expect the channels to open more than once, activation to be mostly over by the peak, and peak probabilities to be relatively high.

One of our central conclusions is that one cannot necessarily identify the rise in probability of the channel being open with activation and the decline with inactivation. Rather, over most of the activation range, the entire process reflects primarily activation, and inactivation is rapid and voltage independent. These observations have obvious implications for underlying mechanisms. If one tries to interpret the molecular basis for sodium channel activity in terms of the traditional activation-inactivation scheme, quantitative if not qualitative errors are certain to result. A specific example of such an error is the suggestion in earlier work from our laboratory that activation and inactivation are independent processes²⁴. The arguments made were based on the assumption that the rate limiting step in the decline of sodium channel probabilities was inactivation whereas, under the conditions of that experiment, this declining

phase was dominated by activation. We now find, based on experiments using a three-pulse protocol¹⁵ that inactivation of closed channels is much slower than that of open channels so that the inactivation process must be coupled to activation.

Our conclusions also simplify the task of understanding sodium channel behaviour by decomposing the entire problem into smaller independent problems. For example, earlier workers have been forced to make a theory that accounts simultaneously for the entire activation-inactivation sequence. Because the sodium channel behaviour can be reconstructed by convolving the first latency densities with the duration distribution function, we can attack the overall problem by treating these two functions independently. For example, one may make a theory for the durations without having to worry about the first passage times to the open state. In fact, because durations are approximately exponentially distributed, a reasonable theory is to assume the existence of only one open state with transitions occurring out of the state according to a Poisson process. Furthermore, most of these transitions are to the absorbing inactivated state. Similarly, one can construct theories for the first passage time to the open state without being concerned with the later behaviour of the channel and the possible existence of, for example, multiple open states.

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Modular arrangement of functional domains along the sequence of an aminoacyl tRNA synthetase

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Gene deletions show that much of Escherichia coli alanine tRNA synthetase is dispensable for each of three activities and that these activities appear to require specific domains arranged linearly along the polypeptide. Thus, variable fusions of extra polypeptide domains to a catalytic core may account for the diverse sizes of aminoacyl tRNA synthetases.

CONSIDERABLE variation in subunit size and quaternary structure is shown by individual members of the aminoacyl tRNA synthetase class of enzymes^{1,2}. This contrasts with the similar reaction catalysed by each of the enzymes and by the small size variation shown by the various tRNA species³. Quaternary structures which have been characterized include α , α_2 , $\alpha_2\beta_2$, and α_4 ^{1,2}. Subunit sizes range from approximately 330 amino acids for the Trp-tRNA synthetase⁴ to lengths over 1,000 amino acids for the isoleucine⁵ and valine enzymes⁶. Because the Trp-tRNA synthetase subunit has a complete set of sites for substrates⁷, the core synthetase necessary for achieving aminoacylation appears to require as little as 300-350 amino acids. This suggests that, for larger synthetases, sizeable sections of the

polypeptide sequence are not for the purpose of aminoacylation. Indeed, additional functions have been proposed for some synthetases, such as regulation of gene expression⁸.

These issues were explored further with *Escherichia coli* Ala-tRNA synthetase, an α_4 tetramer which is the largest of the known synthetases⁹. The entire 875 amino acid sequence was determined previously by sequencing of the coding region of the gene and also by independently establishing the amino acid sequence of large sections of the polypeptide¹⁰. Our purpose was to determine the layout of the functional parts of this polypeptide chain. This was approached by dissecting the polypeptide into pieces and attempting to find, for example, the smallest piece which enables the enzyme to function *in vivo* and

sustain protein synthesis. In this dissection, we also attempted to differentiate the part of the catalytic site required for synthesis of aminoacyl adenylate from the additional segment needed to attach the activated amino acid to tRNA^{Ala}. Finally, an effort was made to find those segments of the polypeptide required for assembly of the protein into a tetramer.

In order to perform this analysis, advantage was taken of the cloned gene and of two strategies for removing specific segments of its coding region. For cases where synthesis of the corresponding truncated polypeptide could be unequivocally demonstrated, functional tests were performed on each one. In this way, it was possible to map functional regions within this large synthetase.

Construction of deletions

Figure 1a is a diagram of plasmid pMJ801. A segment of DNA containing the gene for alanine tRNA synthetase (*alaS*) was cloned into the *Bam*HI site of pBR322. The direction of transcription and span of the coding region of *alaS* are indicated.

All deletions described here were performed on pMJ801. The gene deletions resulted in a nested set of truncated polypeptides. Each starts at the normal amino terminus and extends for different lengths towards the carboxyl terminus of the protein. Deletions were constructed in this way because limited proteolysis of the native protein suggested that at least part of the catalytic activity resides in the amino-terminal half of the protein⁹.

Figure 1b shows a scheme for production of deletions which remove variable amounts of the carboxyl terminal coding region. Plasmid pMJ801 is cut at the *Clal* site and then treated with the Klenow fragment of DNA polymerase I in the presence of 2'-deoxyadenosine 5'-O-(1-thiotriphosphate) (dATP[α S])¹¹. The combined action of the enzyme's exonuclease (in the 3' to 5' direction) and polymerase activities results in replacement of 3'-terminal dAMP with dAMP [α S]¹². The replaced residue is encircled in Fig. 1b. The presence of this nucleotide at the 3'-ends makes the DNA fragment inert to the action of exonuclease III¹¹. However, the side of the fragment adjacent to the *alaS* coding region can be re-exposed by cleavage with *Hind*III. Subsequent treatment with exonuclease III results in asymmetric digestion of just the carboxyl terminal part of the *alaS*-coding region of the DNA fragment. Treatment with S₁ nuclease¹³ and Klenow fragment (in the presence of the four deoxynucleotide triphosphates) gives blunt ends which can be recircularized to give plasmids with variable amounts of the *alaS* C-terminal coding region removed. Because the Amp^r part of the plasmid is left intact, ampicillin-resistant clones can be selected after transformation.

Figure 1c shows a second scheme. With this strategy, fragments of increasing size are removed from the carboxyl-terminal coding region. The plasmid is linearized by limited digestion with either *Msp*I or *Taq*I; these enzymes have four base pair recognition sequences¹⁴ and therefore have multiple sites within the plasmid, but conditions were adjusted so that only one site per molecule was cut. After this limited digestion, the DNA was cut to completion at the unique *Clal* site. Fragments with 1–3 kilobases (kb) deleted were recircularized with T4 DNA ligase. The resulting plasmid mixture was then transformed into *E. coli* with a selection for Amp^r clones.

In both schemes, the TAA stop codon of *alaS* is removed. However, stop codons are present in all three reading frames just beyond the *Clal* site¹⁵ so that the number of residues added to the truncated terminus of Ala-tRNA synthetase is minimized (0–16 amino acids).

Characterization of gene deletions

From the first scheme 78 transformants were analysed and one-third of the recombinant plasmids were found to be intermediate in size between pBR322 and pMJ801. These presumably are deleted in the *alaS* coding region. The remaining ones were either smaller than pBR322 or similar in size to pMJ801. The latter, which comprised less than 20% of the total transformants, may have been untouched by exonuclease III. Of those

Table 1 Properties of amino-terminal fragments of Ala-tRNA synthetase

Size of amino terminal dA/S protein segment (No. amino acids)	Approximate amount synthesized from plasmid	Adenylate synthesis	Aminoacylation of tRNA ^{Ala} (complementation <i>in vivo</i> of <i>alaS5</i>)	Oligomerization
NH ₂ —297 CO ₂ ⁻	m	—	—	n.d.
NH ₂ —385 CO ₂ ⁻	m	+	—	—
NH ₂ —404 CO ₂ ⁻	l	+	—	—
NH ₂ —461 CO ₂ ⁻	m	+	+	n.d.
NH ₂ —468 CO ₂ ⁻	h	+	+	—
NH ₂ —502 CO ₂ ⁻	m	+	+	—
NH ₂ —596 CO ₂ ⁻	m	+	+	n.d.
NH ₂ —612 CO ₂ ⁻	m	+	+	n.d.
NH ₂ —699 CO ₂ ⁻	l	+	+/-	—
NH ₂ —808 CO ₂ ⁻	h	+	+	—
NH ₂ —852 CO ₂ ⁻	m	+	+	+
NH ₂ —875 CO ₂ ⁻ (Native)	h	+	+	+

Protein amounts were estimated from autoradiograms of SDS-polyacrylamide gels of maxicell extracts and, where possible, from measurements of ATP-PP_i exchange activity. Maxicell synthesis is as described in Fig. 2. The pyrophosphate exchange assay was a modification of the procedure of Calendar and Berg²⁰ as described by Putney *et al.*⁹ using either 2 mM alanine or valine (to estimate Val-tRNA synthetase as an internal standard) in the reaction. Crude extracts were prepared from alumina ground cells (strain KL380 containing a deletion plasmid) which were grown in LB medium to log phase ($A_{595} = 1$). Protein concentration was determined by the dye-binding method³⁹ (BioRad). 25 μ g of protein was used in each 1 ml reaction. Aminoacylation of tRNA^{Ala} *in vivo* was checked by testing for complementation of the *alaS5* chromosomal mutation. Plasmids were transformed into the temperature sensitive *alaS5* strain KL380^{22,40}. Oligomerization of truncated proteins was determined as described in Fig. 3. Truncated genes contain C-terminal coding sequences from pBR322 typically for the same 16 amino acids, except for 404(6), 461(14) and 468(4). Symbols used: h, high (8–10-fold amplification of *alaS* protein over levels produced by wild type strain which does not contain plasmid); m, moderate (3–6-fold); l, low (less than 3-fold); +, presence of an activity; —, absence of an activity; ND, not determined.

which were of the desired intermediate size, restriction digests established an approximate location of the deletion end points. These were found reasonably well distributed from the 3'-end to the 5'-end of the *alaS* coding region. Seven were selected for further analysis and these were sequenced (by the dideoxy method^{16,17}) across the deletion end point to determine the exact end of the *alaS* sequence and the number of in-frame pBR322 codons encountered before a stop.

From the second scheme, 40 recombinants were investigated further. These had approximately 1–3 kb deleted, as expected, in a clockwise direction from the former *Clal* site (see Fig. 1c). Restriction digests on these recombinants established the location of the original *Msp*I or *Taq*I site which was cut and ligated to the *Clal* site. This in turn established the exact end point of the deletion and, from the known sequence of pBR322, the number of extraneous codons added before the first in-frame stop codon.

Characterization of truncated polypeptides

Production of proteins from the truncated *alaS* gene segments was investigated in maxicells where plasmid-encoded proteins are synthesized selectively in the presence of ³⁵S-methionine¹⁸. Crude extracts derived from maxicells containing the recombinant plasmids were run on SDS-polyacrylamide gels¹⁹ and *alaS* protein fragment intensities were compared, on an autoradiogram, with that of the plasmid encoded β -lactamase (responsible for Amp^r). Molecular weight standards were used to establish protein sizes. An autoradiogram of a gel containing some of these truncated proteins is shown in Fig. 2.

In all cases where visualization was unequivocal (approximately two-thirds of the recombinants), sizes of the plasmid-

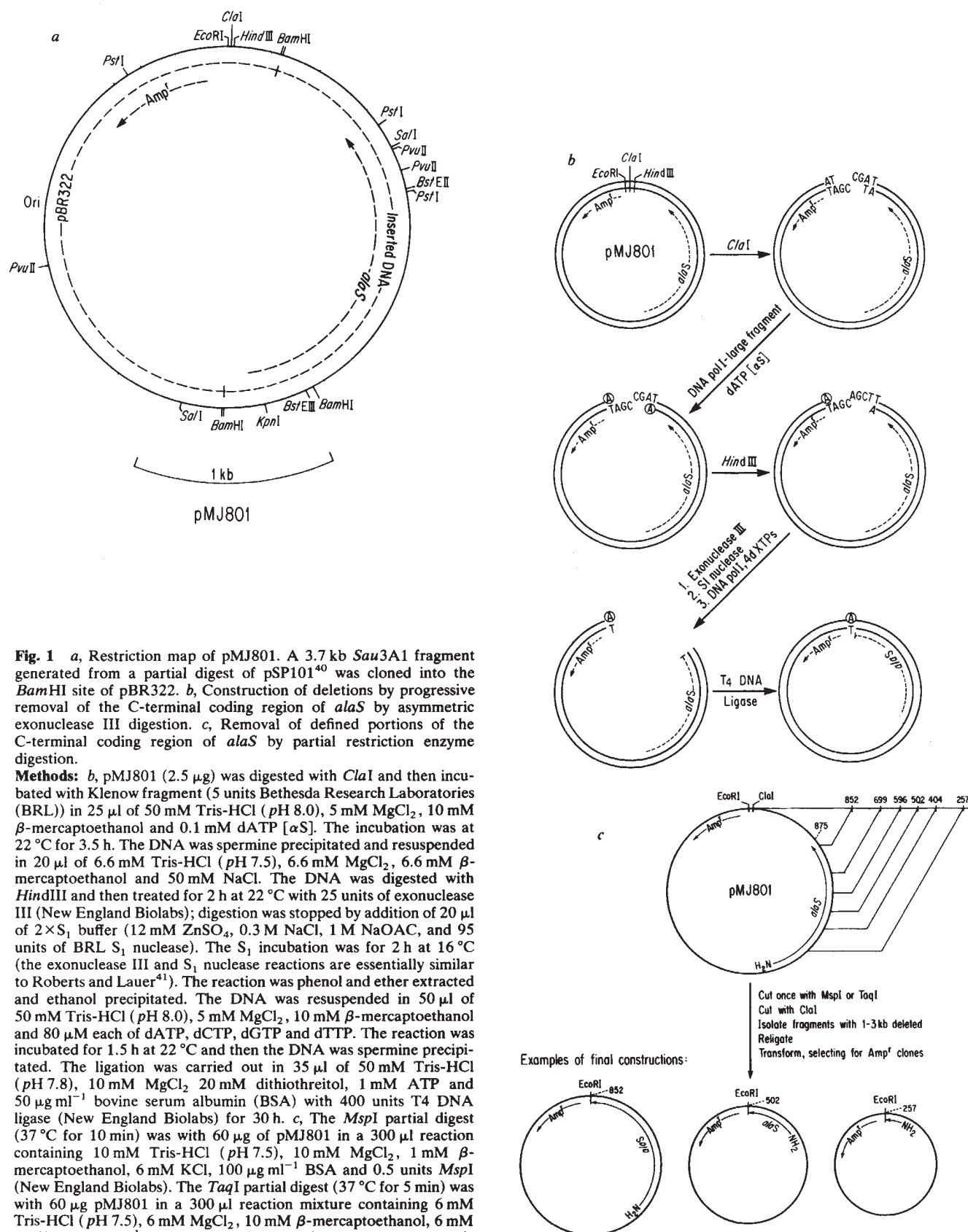


Fig. 1 *a*, Restriction map of pMJ801. A 3.7 kb *Sau3A1* fragment generated from a partial digest of pSP101⁴⁰ was cloned into the *Bam*HI site of pBR322. *b*, Construction of deletions by progressive removal of the C-terminal coding region of *alaS* by asymmetric exonuclease III digestion. *c*, Removal of defined portions of the C-terminal coding region of *alaS* by partial restriction enzyme digestion.

Methods: *b*, pMJ801 (2.5 μ g) was digested with *ClaI* and then incubated with Klenow fragment (5 units Bethesda Research Laboratories (BRL)) in 25 μ l of 50 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 10 mM β -mercaptoethanol and 0.1 mM dATP [α S]. The incubation was at 22 °C for 3.5 h. The DNA was spermine precipitated and resuspended in 20 μ l of 6.6 mM Tris-HCl (pH 7.5), 6.6 mM MgCl₂, 6.6 mM β -mercaptoethanol and 50 mM NaCl. The DNA was digested with *HindIII* and then treated for 2 h at 22 °C with 25 units of exonuclease III (New England Biolabs); digestion was stopped by addition of 20 μ l of 2 $\times$ S₁ buffer (12 mM ZnSO₄, 0.3 M NaCl, 1 M NaOAc, and 95 units of BRL S₁ nuclease). The S₁ incubation was for 2 h at 16 °C (the exonuclease III and S₁ nuclease reactions are essentially similar to Roberts and Lauer⁴¹). The reaction was phenol and ether extracted and ethanol precipitated. The DNA was resuspended in 50 μ l of 50 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 10 mM β -mercaptoethanol and 80 μ M each of dATP, dCTP, dGTP and dTTP. The reaction was incubated for 1.5 h at 22 °C and then the DNA was spermine precipitated. The ligation was carried out in 35 μ l of 50 mM Tris-HCl (pH 7.8), 10 mM MgCl₂, 20 mM dithiothreitol, 1 mM ATP and 50 μ g ml⁻¹ bovine serum albumin (BSA) with 400 units T4 DNA ligase (New England Biolabs) for 30 h. *c*, The *MspI* partial digest (37 °C for 10 min) was with 60 μ g of pMJ801 in a 300 μ l reaction containing 10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM β -mercaptoethanol, 6 mM KCl, 100 μ g ml⁻¹ BSA and 0.5 units *MspI* (New England Biolabs). The *TaqI* partial digest (37 °C for 5 min) was with 60 μ g pMJ801 in a 300 μ l reaction mixture containing 6 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 10 mM β -mercaptoethanol, 6 mM NaCl, 100 μ g ml⁻¹ BSA and 5 units *TaqI* (New England Biolabs). The partial digests were stopped by the addition of EDTA (pH 8.0) to a final concentration of 10 mM. After ethanol precipitation, both partial *MspI* and *TaqI* digests were cut to completion with *ClaI*. The *ClaI* digests were run on a 0.9% preparative agarose gel. Fragments between 4.7 and 7 kb were eluted from the agarose. Approximately 5 μ g of DNA was recovered for each digest. Ligation of 1.25 μ g *MspI/ClaI* or of *TaqI/ClaI* fragments was as described above in a 100 μ l reaction (4 °C for 12 h). After ethanol precipitation the ligated DNA was digested with *HindIII*.

encoded proteins on SDS gels were consistent with those expected by determination of deletion end points. A summary is given in Table 1. The amounts of fragments synthesized varied from quantities similar to that of the native protein to about 10% of that amount (amounts of native enzyme synthesized from the multi-copy plasmid are about 10 times the levels made from the chromosomal gene). Note that, for example, abundant quantities of a 699 amino acid *alaS* protein fragment are made in maxicells, but one with four less amino acids is barely visible on autoradiograms. Thus, small changes in the size of the *alaS* coding region can have a profound effect on protein levels. It is not known if the variation in protein level is due to protein instability, to message instability, or to some other factor.

Where possible, an enzymatic assay was also used to estimate amounts of *alaS* protein fragments. The alanine-dependent ATP-PP_i isotope exchange assay²⁰ is a sensitive measure of the amounts of *alaS* protein fragment which can carry out the adenylate synthesis reaction. For cases (385 amino acids and larger, see below) where the site for adenylate synthesis was not destroyed, levels of alanine-dependent ATP-PP_i exchange were compared with that of the host strain. Activities were normalized by making use of the internal valine-dependent ATP-PP_i exchange of the host strain. There is good correlation between the enhanced alanine-dependent ATP-PP_i exchange and the amount of protein fragment observable on an autoradiogram of the corresponding maxicell extract.

Adenylate synthesis

Aminoacyl tRNA synthetases catalyse aminoacylation in a two-step reaction^{1,2}. In the first reaction, amino acid is condensed with ATP to give an aminoacyl adenylate. This reaction occurs in the absence of tRNA and can be assayed by the ATP-PP_i exchange reaction mentioned above.

A summary of the truncated proteins investigated, and the presence or absence of adenylate synthesis activity measured *in vitro*, is given in Table 1. Starting at the amino terminus, these range in length from 257 to the full 875 amino acid residues of alanine tRNA synthetase. The smallest *alaS* protein (257 amino acids) is devoid of all activity. However, a fragment of only 385 residues is sufficient to give adenylate synthesis activity. We estimate that the specific activity for adenylate synthesis by this fragment is within a factor of two of that of the native protein. Every protein fragment which extends beyond residue 385 also has adenylate synthesis activity. Therefore, residues beyond 385 are neither required for nor do they interfere with adenylate synthesis. The implication is that the adenylate synthesis section of the polypeptide is functionally independent of the rest of the chain.

These results confirm and extend those obtained by proteolysis of native Ala-tRNA synthetase. Mild trypsin treatment gives an N-terminal fragment of molecular weight (MW) 48,000 which has been purified and shown to have full activity for alanyl adenylate synthesis⁹. Further digestion of this fragment gives an N-terminal 40,000 MW fragment which has partial adenylate synthesis activity¹⁰.

tRNA-dependent step

An independent assay can measure the overall aminocyclation of tRNA²¹. This overall reaction is essential to maintain protein synthesis *in vivo*. Because Ala-tRNA synthetase is an essential protein, the ability of a truncated synthetase to sustain cell growth shows directly that it has catalytic sites for aminoacylation. For maximum sensitivity of testing for aminocyclation *in vivo*, we used a temperature-sensitive, conditional lethal host strain which has low levels of Ala-tRNA synthetase activity. The rationale for use of this host is that only a small increment of aminoacylation activity by the truncated protein is necessary to achieve complementation. In this way we could detect small specific activities contributed by the deletion polypeptides.

For this purpose, tests for aminoacylation were done in the *alaS5* background. This host grows well at 30 °C and not at all

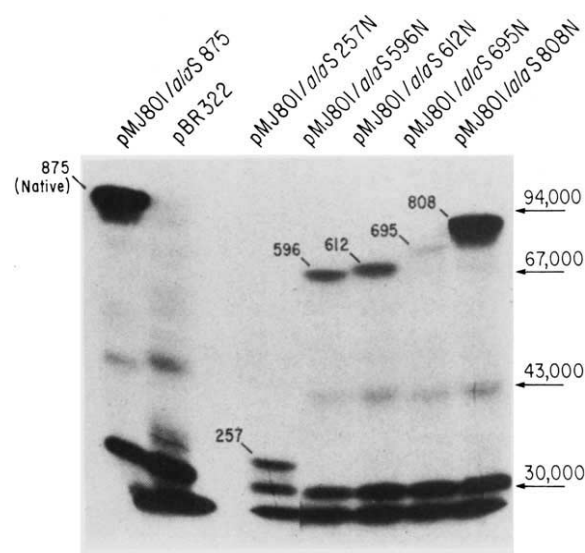


Fig. 2 Autoradiogram of a 10% SDS-polyacrylamide gel of maxicell synthesized proteins from cells containing various deletion plasmids. Deletion plasmids were transformed into a *met⁺ recA⁻* strain (SG4051). Cells were UV-irradiated, incubated overnight with cycloserine, and protein synthesis was allowed to occur in the presence of ³⁵S-methionine¹⁸. The deletion plasmids are designated according to the number of NH₂-terminal *alaS* codons present. Protein products electrophorese at expected positions except near the front (band at bottom of each lane). See Table 1 for further details.

at 42 °C²². Although synthesis of aminoacyl adenylate by the *alaS5* mutant protein appears normal²³, it has a substantially reduced specific activity for tRNA aminoacylation and this is insufficient to maintain cell growth at the restrictive temperature. On a gel filtration column, the enzyme runs as a completely dissociated monomer (MW 95,000), which means that the mutation affects the oligomerization process as well as the tRNA-dependent step in catalysis²³. The use of a mutant allele which shows no subunit association *in vitro* decreases the possibility of complementation via hybrid interactions between deletion polypeptides and the mutant host protein. As shown below, this possibility is further reduced for many of the deletion polypeptides because they have lost the domain for subunit association. Further confirmation was achieved by use of a specially-constructed *alaS* deletion strain which can only be maintained if the plasmid encoded truncated polypeptide alone aminoacylates *in vivo*²³.

Table 1 shows that extending the polypeptide from amino acid 385 to 404 is not sufficient to sustain protein synthesis *in vivo*. However, the protein containing 461 synthetase residues complements the *alaS5* mutation. Therefore, addition of only 76 residues to the part required for adenylate synthesis is sufficient to give the complete catalytic function of the synthetase. This is only slightly over half the full length of the native protein. (Sizes greater than 461 amino acids also show complementation.) Therefore, there are over 400 carboxyl terminal residues which are not required for the basic catalytic activity of the protein. *In vitro* aminoacylation assays indicate that the specific activity for aminocyclation of the 461 residue truncated protein is reduced by at least 5-fold, however.

Complementation was problematical for only one of the polypeptides which is greater in length than 461 amino acids. This is the 695 amino acid fragment whose level is low. Cells containing this recombinant grow poorly at 42 °C. We attribute this to the low levels of stable protein which are present. (Conceivably, it is the low levels of stable protein which prevent the 404 residue chain from complementing, rather than its lack of sites required for the tRNA-dependent step.)

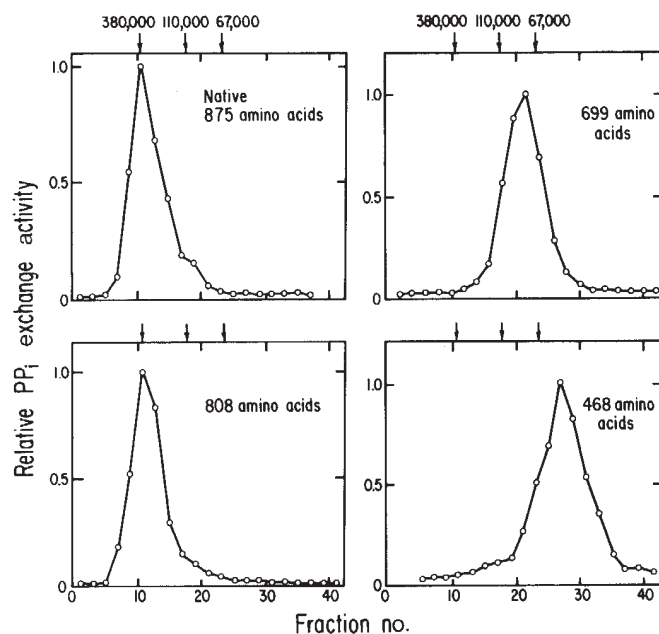


Fig. 3 Column profiles of crude cell extracts containing the deletion plasmids. One mg of protein (see Table 1) was run on a 1×45 cm Sephadex G-200 column equilibrated and eluted with 20 mM KPO_4 (pH 7.5), 100 mM NaCl. Approximately 0.5-ml fractions were collected and these were assayed for alanine and valine dependent ATP-PP_i exchange activity. Graphs only show the amplified alanine-dependent activity with the peak for valine activity indicated by the arrow at 110,000. The position of the MW markers phosphorylase A (380,000) and BSA (67,000) are also shown. The contribution of the unamplified host *alaS5* protein is seen as a small activity at a MW of 110,000.

Further confirmation was obtained by use of an *alaS* deletion mutant in which the gene has been removed from the chromosome; cell growth is then only possible when recombinant plasmids are introduced which direct synthesis of polypeptides with sufficient Ala-tRNA synthetase activity²³. With this host, for example, we achieved complementation with the 468 amino acid and 699 amino acid polypeptides, but not with the 695 amino acid species. Thus, while the latter will barely sustain growth at the restrictive temperature of the *alaS5* host, there are insufficient quantities of stable protein to sustain the deletion strain.

For the fragment containing 461 residues to function *in vivo*, it must not only be fully functional for catalysis, but must also retain the specificity of aminoacylation. *In vitro* assays have shown that extensive mis-aminoacylation occurs when synthetases are altered by the presence of small quantities of organic solvents in a reaction medium^{24,25}. Such mis-acylations would be lethal *in vivo*. Whatever alterations in the chain are caused by the removal of over 400 carboxyl-terminal residues, the remaining fragment has not been altered enough to alter substantially its catalytic specificity.

The results in Table 1 show that the carboxyl-terminal side of the adenylylation synthesis domain is located no further than residue 385. The amino-terminal side of this domain is not defined by the experiments, but the results unequivocally show that residues between 257 and 385 are essential. In the same vein, residues between 404 and 461 are essential for interactions with tRNA to achieve aminoacylation. It is of interest to note that this segment is adjacent in the sequence to that required for adenylylation synthesis. While during catalysis the site for adenylylation synthesis must come spatially close to the 3' end of the tRNA where amino acid attachment occurs, there is *a priori* no requirement for these regions also to be juxtaposed in the sequence.

Tetramer assembly

As shown previously, Ala-tRNA synthetase is an α_4 tetramer⁹. The approximately 400 amino acid NH_2 -terminal fragment obtained on proteolysis is a monomer^{9,10} and this suggests that determinants in the chain for tetramer assembly are located in the carboxyl-terminal half of the protein.

The capacity for oligomerization of some of the truncated proteins listed in Table 1 was tested by gel filtration chromatography in non-denaturing conditions. Results of chromatography on a Sephadex G-200 column are shown in Fig. 3. The column was calibrated with molecular weight standards. As expected, the native protein runs as a tetramer with a MW of 380,000. The deletion containing 808 amino acids also runs as a tetramer on this column. However, the 699 amino acid protein clearly runs as a monomer. Also shown are results with the amino-terminal 468 amino acid fragment; this too runs on the column as a monomer. A more complete summary is given in Table 1.

The results in Fig. 3 show that sites essential for tetramer assembly are contained between amino acids 699 and 808. It is also clear from the foregoing analysis that these residues are separated from those required for catalysis. Moreover, it appears that aminoacylation and maintenance of protein synthesis *in vivo* do not require tetramer assembly. While we cannot exclude the possibility that, for example, the 468 or 699 amino acid fragments oligomerize *in vivo*, there is no evidence *in vitro* for even a small amount of tetramer formation. As discussed below, tetramer formation is believed to be essential for another biological function of this synthetase.

Figure 4 summarizes conclusions obtained from the nested set of C-terminal deletions. The C-terminal limits of domains for each of three functions is shown by shading. No more than the first 461 amino acids are required to give a stable protein which aminoacylates tRNA *in vivo*. Thus, the 'core' enzyme can function without the C-terminal half.

If the C-terminal piece is fused to the core enzyme to impart an additional function, then, at least to some degree, this C-terminal segment should function independently. This possibility was tested by creation of a large internal gene deletion in the section which encodes the catalytic domains (Fig. 5). The 2,625 nucleotide coding region was opened at the *Bgl*II site (nucleotide 631) and then partially digested with *Bal*31 nuclease²⁶. From subsequent analyses, an internal deletion was isolated which joins codon 66 to codon 351 (shown by DNA sequencing^{16,17}), so that 285 internal amino acids have been removed. Examination of SDS-polyacrylamide gels of extracts, from maxicells containing this deletion construction, showed synthesis of the expected 65,000 MW polypeptide. Although catalytically inactive, it was precipitated by polyclonal antibodies directed against Ala-tRNA synthetase^{27,28}, thus confirming that it is the expected *alaS* internal deletion protein.

Gel filtration on a Sephadex G-200 column, which was calibrated with several molecular weight standards, showed that the *alaS* internal deletion protein runs at a MW of 240,000 (Fig. 5). Thus, oligomerization occurs even with a major disruption of the amino-terminal half which contains the core enzyme.

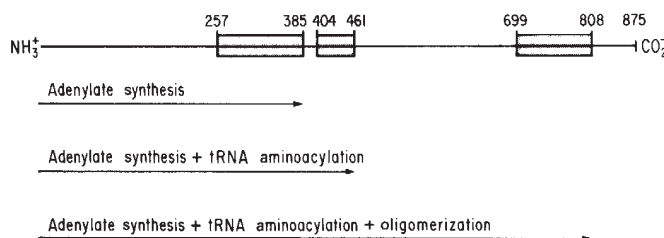
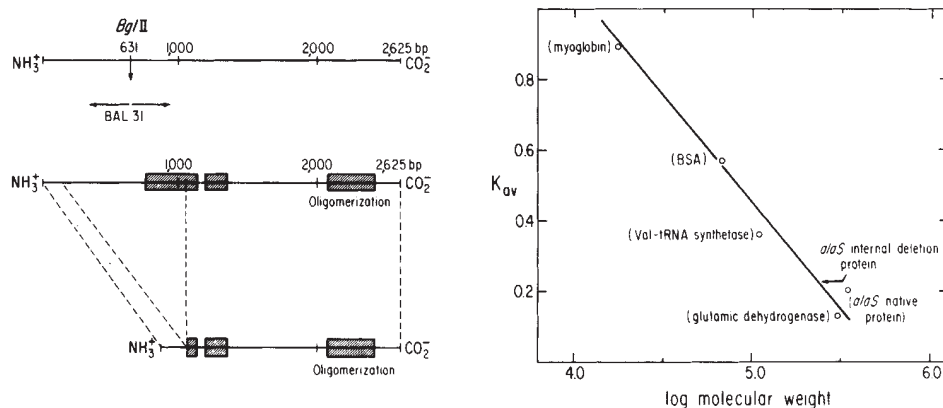


Fig. 4 Regions essential for structure and function of Ala-tRNA synthetase. Shaded areas bracket the C-terminal boundaries of residues required for protein fragments with a specific function.

Fig. 5 Creation of an internal deletion in *alaS* which preserves the domain required for oligomerization. The *alaS* deletion protein was analysed as follows. A protein extract was prepared from a 2.5-litre mid-log culture of SG4051 carrying the internal deletion plasmid. The plasmid-encoded proteins were preferentially labelled with ^{35}S -methionine, using the maxicell procedure¹⁸. The protein extract was chromatographed using a G-200 gel-filtration column, with 20 mM potassium phosphate (pH 7.5), 50 mM sodium chloride, 1 mM phenylmethylsulphonyl fluoride (PMSF) as column buffer. The column fractions were assayed for alanine- and valine-dependent PP_i exchange activity⁹ to locate the chromosome encoded Val-tRNA synthetase and Ala-tRNA synthetase as internal molecular weight markers. The column was also calibrated using a series of protein standards, as shown. The data were plotted as log molecular weight versus K_{av} , where $K_{av} = (V_e - V_0)/(V_t - V_0)$ and V_e , V_0 and V_t are the elution volume, void volume and total column volume respectively. To locate the internal deletion protein column fractions were dialysed against 1 mM PMSF in distilled water, lyophilized, resuspended in sample buffer and electrophoresed on 10% SDS polyacrylamide gels¹⁹. The ^{35}S -labelled, internal deletion protein was then visualized by autoradiography of these gels.



Conclusions

Limited proteolysis of native Ala-tRNA synthetase gives rise to a 48,000 MW and subsequently to a 40,000 MW monomeric N-terminal fragment^{9,10}. While the carboxyl termini of these proteins have not been sequenced, the size of the latter corresponds approximately to the C-terminal boundary of the adenylate functional region shown in Fig. 4. This suggests that the functional domain corresponds to a structural domain. More recent experiments, at shorter times of digestion, have indicated the appearance of two or three discrete intermediates at higher molecular weights which can be correlated with locations of some of the other regions in Fig. 4²⁹. However, these intermediates are mixed together and have only been resolved on denaturing gels, so that further characterization has not yet been possible.

As stated earlier, the core synthetase may be as small as 300–350 amino acids, a size which corresponds to that of the Trp-tRNA synthetase subunit⁴. Figure 4 shows that a 461 amino acid fragment of Ala-tRNA synthetase contains enough information to aminoacylate tRNA *in vivo*. This size is not much larger than that of the Trp-tRNA synthetase.

The methionine enzyme is an α_2 protein whose subunit size is approximately 800 amino acids³⁰. About 200 amino acids may be removed by proteolysis from the carboxyl terminus to give a roughly 600 amino acid truncated polypeptide^{30,31}. This protein does not dimerize but does carry out aminoacylation^{30–32}. While extensive studies have not been carried out, these initial findings on the methionine system suggest that a considerable amount of polypeptide sequence is devoted to functions other than aminoacylation.

High resolution X-ray crystallographic data are available on two synthetases: *Bacillus stearothermophilus* Tyr-tRNA synthetase³³ and the aforementioned fragment of *E. coli* Met-tRNA synthetase^{34,35}. Two tandem structural domains are found in the 419 amino acid tyrosine enzyme. The first, involving the amino-terminal 220 residues, consists of a mononucleotide-binding fold which forms the site for tyrosyl adenylate. This is followed by a domain composed of five α -helices. The large fragment of the methionine enzyme has three structural domains. The first, which encompasses about 250 residues which begin at the N-terminus, forms a mononucleotide-binding fold. Note that adenylate synthesis requires no more than the N-terminal 385 amino acids of the alanine enzyme (Fig. 4) and, by analogy with the tyrosine and methionine synthetases, this domain may be arranged into a mononucleotide-binding fold. Previously noted sequence homologies^{10,36} between these three enzymes, and a site-specific mutation which affects ATP bind-

ing³⁷, all fall in the 'adenylate' domain of the respective proteins.

The organization of Ala-tRNA synthetase suggests that the great diversity in subunit sizes of synthetases, and in quaternary structures², is due to the fusion of additional functional domains to the core polypeptide. These fusions, in principle, may occur at the amino- or at the carboxyl-terminal side of the core enzyme and, therefore, the core enzyme in each case may start at a different distance from the amino terminus. In general, in comparing sequences between synthetases, the presence of additional functional domains in some of the enzymes means that only a fraction of the sequence can be used for meaningful structural comparison and that the relevant catalytic parts may be located very different distances from the respective amino termini. Both of these possibilities are consistent with the sequence comparisons done so far^{10,36,38}.

Chemical cross-linking of the native Ala-tRNA synthetase tetramer, followed by denaturation and dissociation, shows the presence of dimers and tetramers in the cross-linked population⁹. This argues for a subunit arrangement which generates 2-fold symmetry in the tetramer⁹. The enzyme is known to repress its gene transcription *in vitro* by binding to an interrupted palindrome which flanks the promoter site⁸. This implies that a 2-fold axis in the protein fits onto that of the DNA. Therefore, the oligomerization domain is most probably associated with an additional function of this synthetase—that of gene regulation. This domain may in turn have been added on to that for the core enzyme.

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Crystal structure of leucine-enkephalin

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The crystal structure of leucine-enkephalin has been determined in a crystal form that has four independent enkephalin molecules and much water and dimethylformamide solvent in the asymmetric unit. All four enkephalins have extended peptide backbones with the Tyr, Phe and Leu side chains above and below the plane of the backbone. There is evidence that this extended conformation may provide an acceptable model for enkephalin binding to opiate μ -receptors.

THE search for endogenous brain substances which bind to opiate receptors culminated several years ago in the isolation and characterization by Hughes *et al.*¹ of the pentapeptides leucine (Leu)-enkephalin (Tyr-Gly-Gly-Phe-Leu) and methionine (Met)-enkephalin (Tyr-Gly-Gly-Phe-Met). An understanding of how the enkephalins effect their functions and the establishment of guidelines for the development of potent and selective analogues depend heavily on knowledge of enkephalin three-dimensional stereochemistry, and attempts to obtain conformational data soon followed. High-resolution NMR spectra were interpreted by two groups^{2,3} as indicative of a solution conformation characterized by a β -bend between Gly 3–Phe 4, stabilized by Gly 2 (carbonyl)–Met 5 (or Leu 5) (amide) hydrogen bonding; a third study⁴, however, found no basis for intramolecular hydrogen bonding. Some of the conflicting results were explained by Khaled *et al.*⁵, who demonstrated by NMR, UV and circular dichroism studies that the spectral properties of enkephalins which are characteristically used to evaluate conformation differed at different concentrations. They postulated two conformations, which are both consistent with the NMR data: a structure with a Gly 3–Phe β -bend, similar to the above but with additional hydrogen bonding, and extended conformation molecules associated by intermolecular hydrogen bonds to form an antiparallel β -pleated sheet structure.

Model building experiments led Bradbury *et al.* to propose⁶ a Met-enkephalin conformation having a β -bend between Gly 2 and Gly 3 and a hydrogen bond between Tyr 1 (C=O) and Phe 4 (NH). Empirical energy calculations of some possible conformations of Met-enkephalin⁷ and analogues⁸ resulted in models having Gly 3–Phe 4 bends in common but different backbone and side-chain arrangements. Another conformational analysis⁹ found little energy differences between fully extended structures and several folded (various types of β -turns between Gly 2–Gly 3 or Gly 3–Phe 4) ones.

The first X-ray diffraction structural information came from a crystal structure investigation¹⁰ of Leu-enkephalin monohydrate, in crystals grown from aqueous methanol. The molecular conformation is characterized by a Gly 2–Gly 3 β -bend and a Tyr 1 (carbonyl)–Phe 4 (amide) hydrogen bond, similar to that proposed from empirical model-building⁶, but with an addi-

tional intramolecular hydrogen bond from Tyr 1 (NH) to Phe 4 (C=O). The Tyr and Phe side chains are located on the same side of the molecular backbone 'plane' and are roughly coplanar with it, while the Leu side chain is on the opposite side and oriented perpendicularly to the backbone. Subsequently¹¹ it was discovered that the unit cell of the Leu-enkephalin monohydrate crystals had been incorrectly characterized; the true cell contains four independent peptide molecules and the results of the structure analysis can be interpreted as describing a conformation averaged over four nearly identical molecules. Refinement of the true crystal structure has so far been unsuccessful (T. L. Blundell, personal communication).

A significant advance in the delineation of conformational requirements for enkephalin opioid activity was achieved with the synthesis of a semi-rigid cyclic analogue, Tyr-cyclo [$-N^{\gamma}$ -D-diaminobutyric acid-Gly-Phe-Leu] (ref. 12), with high potency in opiate μ -receptor biological and binding assays^{12,13}. Comparative results with modified derivatives strongly suggested that the modes of binding of cyclized and open-chain analogues are identical. The ring structure greatly limits the conformational variability of the cyclized analogue, and in particular does not allow formation of a β -bend; thus, most of the conformational features proposed for enkephalin from the theoretical and solution spectroscopic studies, as well as the conformation observed in the Leu-enkephalin monohydrate crystal, cannot be realized in the cyclic analogue and therefore cannot represent the stereochemistry of enkephalin binding to μ -opiate receptors.

We have grown crystals of Leu-enkephalin from *N,N*-dimethylformamide (DMFA)–water solution, collected a high-resolution X-ray diffraction data set, and elucidated the crystal and molecular structures. The crystal form is different from that previously investigated, and contains four independent Leu-enkephalin molecules and a considerable number of solvent molecules, both DMFA and H₂O, in the asymmetric unit. We now present the results of the structure determination. The molecules observed in this crystal form have extended conformations which are consistent with NMR spectra, theoretical calculations and with stereochemical characteristics of the active cyclized analogue; hence, the observed structures may fit the conformational requirements for opiate μ -receptor binding.

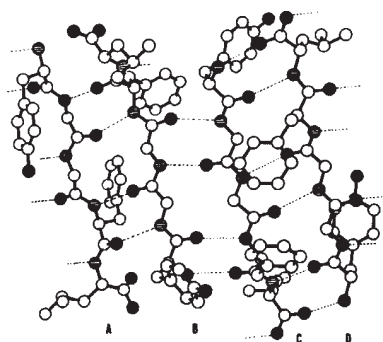


Fig. 1 Conformations of the four independent pentapeptides comprising the Leu-enkephalin crystal structure asymmetric unit. Oxygen atoms are blackened and nitrogen atoms are striped. Hydrogen bonds are represented by dotted lines.

Experimental methods

Crystals of Leu-enkephalin, grown from a mixture of DMFA and water, give up solvent of crystallization on standing and lose their crystallinity. Consequently, for collection of X-ray data, a single crystal was sealed in a capillary tube with mother liquor present. X-ray photographs and diffractometer measurements indicate space group $P2_1$ with unit cell dimensions $a = 18.720$, $b = 24.732$, $c = 20.311$ Å, $\beta = 115.86^\circ$. Density considerations suggested the asymmetric unit to consist of four enkephalin molecules plus 40–50 solvent atoms. X-ray intensity data, collected on a four-circle diffractometer to a resolution of $2\theta = 110^\circ$ with copper radiation, totalled 10,942 independent reflections, of which 5,965 had an intensity greater than twice the standard deviation of the measurement.

The structure was solved by use of the symbolic addition procedure¹⁴ in a non-routine fashion. Forty peaks consistent with an antiparallel β -pleated sheet arrangement were selected from an E-map generated with phases determined from the Σ_2 and Σ_3 relationships. Iterative applications of various procedures—tangent formula for phase extension, atom placement from chemical structure considerations, calculation of difference electron-density maps and restrained least-squares refinement of partial structure—together with relaxation of space group symmetry for correct origin determination, eventually led to the location of 213 atoms in the asymmetric unit: four Leu-

Table 1 Leu-enkephalin hydrogen-bonding pattern

	A	B	C	D
Tyr NH ₂	A', D, W	B', C, W	D', D, C'	D', C, D'
Tyr CO	D	C	D	C
Gly NH	B	A	B	A
Gly CO	B	A	B	A
Gly NH	D	C	D	C
Gly CO	D	C	D	C
Phe NH	B	A	B	A
Phe CO	B	A	B	A
Leu NH	D'	C'	D	C
Leu CO ₂ H	A'W	B'W	B', D, C'	A', C, D'

The table should be read as: tyrosine amino group of molecule A is hydrogen bonded to molecules A' and D and to a water molecule.

enkephalin, eight water and nine DMFA molecules. Complete details of crystal structure determination are presented elsewhere¹⁵. Restrained least-squares refinement^{16,17} of 160 Leu-enkephalin non-hydrogen atoms, 53 water and DMFA atoms and 108 enkephalin hydrogens reduced the discrepancy index R to 0.119 for 8,155 data with $|F| > 0$ and weighted R to 0.079 for 9,081 data.

Crystal structure

The four independent Leu-enkephalin molecules in the asymmetric unit form a slightly irregular antiparallel β -pleated sheet as shown in Fig. 1. The pleated sheet is roughly parallel to the crystallographic ac plane with the amino acid side chains oriented alternately above and below the plane. The four molecules, labelled A, B, C and D in Fig. 1, are held together by hydrogen bonds largely between peptide backbone NH and CO groups. Molecules C and D also share links between their terminal amino and carboxyl groups whereas the equivalent atoms of A and B are hydrogen bonded not to each other but to other enkephalin molecules and to water. The overall symmetry of the hydrogen bonding and the small differences in bonding patterns between A–B and C–D are illustrated in Table 1. As the table shows, the β -pleated sheet is not limited to the four molecules comprising the asymmetric unit, but is continued in both the crystallographic a and c directions through hydrogen bonding between repeat units (links between molecules D and A in one crystal direction and between A–A', B–B', C–B', C–C', D–A' and D–D' in the other). This hydrogen-bonding scheme is shown more clearly in Fig. 2 which illustrates stereoscopically

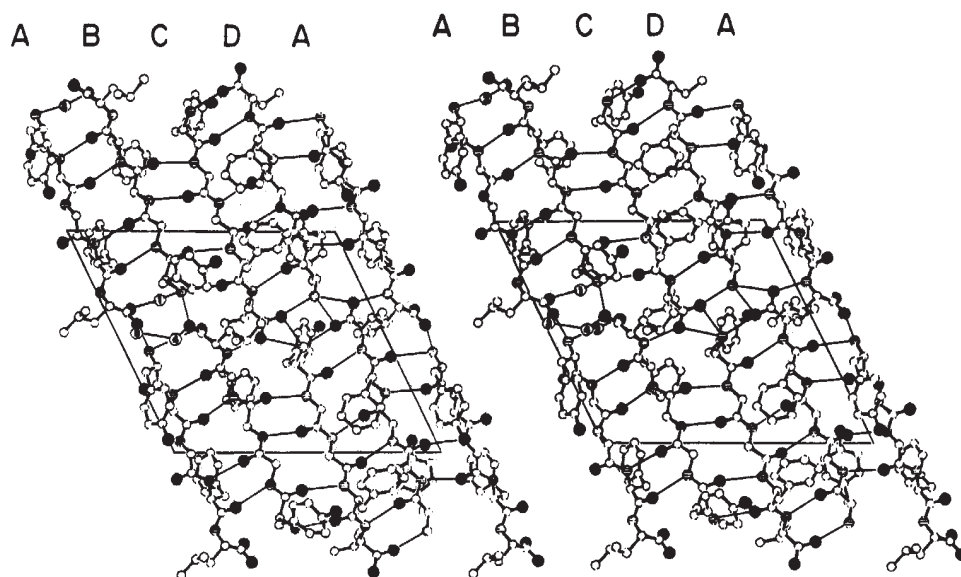
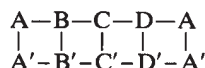


Fig. 2 Stereoscopic drawing illustrating the hydrogen bonding interactions between Leu-enkephalin molecules forming a two-dimensional infinite antiparallel β -pleated sheet. Oxygen atoms are blackened, nitrogen atoms are striped and water molecules are numbered. Hydrogen bonds are represented by solid lines.

the arrangement of enkephalin molecules



Thus, the crystal structure of Leu-enkephalin in this crystal form is a two-dimensional infinite antiparallel β -pleated sheet formed by the enkephalin peptide backbones in extended conformations, with the Tyr, Phe and Leu side chains located above and below the backbone plane. Because of the crystallographic 2_1 screw axis, the two-dimensional sheet is repeated at a distance of $b/2$ in the third dimension, but the separation is 12.36 Å and there are no direct contacts between side chains of different enkephalin molecules. Rather, tyrosine hydroxyls are hydrogen bonded to solvent molecules that fill the spaces between the sheets of peptide molecules: A and D to DMFA molecules, B and C to water molecules. Some of the DMFA molecules are ordered and anchored in these spaces by hydrogen bonds to other solvent molecules and some apparently do not take part in hydrogen bonding and are disordered. Detailed solvent interactions are presented elsewhere¹⁵.

Molecular structure

The four enkephalins in the asymmetric unit exhibit somewhat different molecular conformations but, significantly, all have extended linear backbones. This is in marked contrast to most of the previous studies, both experimental and theoretical, which have postulated or found Leu-enkephalin to adopt a hairpin conformation with a β -bend stabilized by intramolecular hydrogen bonding.

The molecular conformations of the four independent enkephalins are compared in two views in Fig. 3. The greatest differences between the four molecules are the orientations of the Tyr and Leu side chains; the Phe side-chain orientation, in

Table 2 Torsion angles (deg) in the enkephalin backbones

Residue	Angle	A	B	C	D
Tyr 1	ψ_1	135	154	155	137
	ω_1	172	177	177	173
Gly 2	ϕ_2	-144	151	141	-131
	ψ_2	114	-155	-157	142
Gly 3	ω_2	-177	180	-178	179
	ϕ_3	-122	154	174	-144
Phe 4	ψ_3	132	-151	-170	131
	ω_3	-179	-170	179	178
Phe 4	ϕ_4	-122	-128	-119	-147
	ψ_4	139	130	149	152
Leu 5	ω_4	168	174	179	171
	ϕ_5	-79	-72	-141	-141
	ψ_5	176	167	137	151

Estimated standard deviations are 1.8–2.5°.

contrast, differs only slightly from molecule to molecule. The peptide backbones of A and D are similar and are somewhat more puckered (less flat) than are those of B and C, which also resemble each other. The tyrosine rings of A and D are similar in orientation, as are those of B and C; the orientations differ by rotation about the tyrosine $C^\alpha-C^\beta$ bond. In all four molecules the regular alternation of side-chain positions causes the Tyr and Phe side chains to be located on opposite sides of the backbone, with the Leu side chain on the same side as the Tyr. This arrangement is different from that found in the previous Leu-enkephalin β -bend crystal structure in which the Tyr and Phe rings were located on the same side and the Leu on the other side of the peptide backbone.

Molecular structure and biological activity

One explanation for the range of enkephalin conformations postulated from spectroscopic and theoretical analyses is that these pentapeptides are flexible and capable of assuming more than one shape in solution. Indeed, differences between the four independent Leu-enkephalin molecules in this crystal structure clearly show that the Tyr, Phe and Leu side chains can assume different orientations even in the same crystal lattice. In addition, contrasting the extended conformations of the four enkephalin molecules with the folded conformation found in the crystal form obtained from aqueous methanol¹⁰ presents conclusive evidence of the ability of the peptide backbone to maintain greatly differing conformations, depending, presumably, on environmental factors.

The enkephalin conformation with the peptide backbone fully extended is apparently stable in many environments. Calculations on enkephalin isolated molecules⁹ concluded that the extended conformation is energetically favoured while spectroscopic data for enkephalins in solution⁵ are consistent with molecules with linear backbones associated as dimers in an antiparallel β -sheet arrangement. This crystal structure determination demonstrates that in a highly water/DMFA solvated solid state environment the extended conformation is also favoured, with the monomers arranged in an antiparallel pleated sheet as suggested by the spectroscopic data, but with the sheet infinite in two dimensions rather than limited to a dimeric association. The manner in which monomers are linked in the infinite sheet provides examples, at the molecular level, of the patterns of hydrogen-bonding interactions that the enkephalins could make with receptors and with other solvent molecules.

As mentioned in the description of enkephalin molecular structure, the peptide backbones of molecules A and D are more puckered than are those of molecules B and C. The magnitudes of these conformational variations are shown in Table 2, which lists the backbone torsion angles in the four molecules. Analysis of the ϕ and ψ angles for glycine residues 2 and 3 reveals that at these positions molecules A and D display conformations characteristic of L-amino acids while for B and C the values are closer to those which would be favoured by

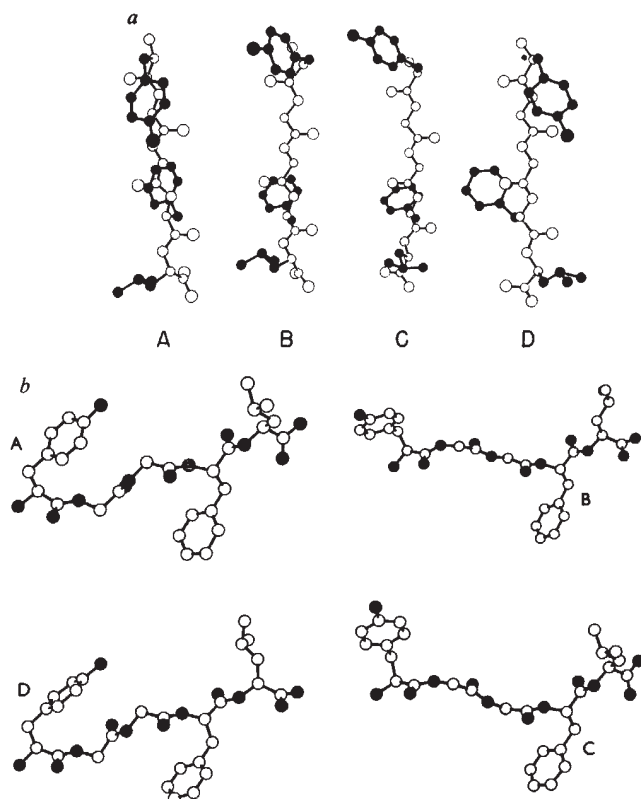


Fig. 3 *a*, Conformations of the four Leu-enkephalin molecules aligned in comparable orientations. Oxygen atoms are blackened and nitrogen atoms are striped. *b*, An alternative view. Tyrosine, phenylalanine and leucine side chains are blackened.

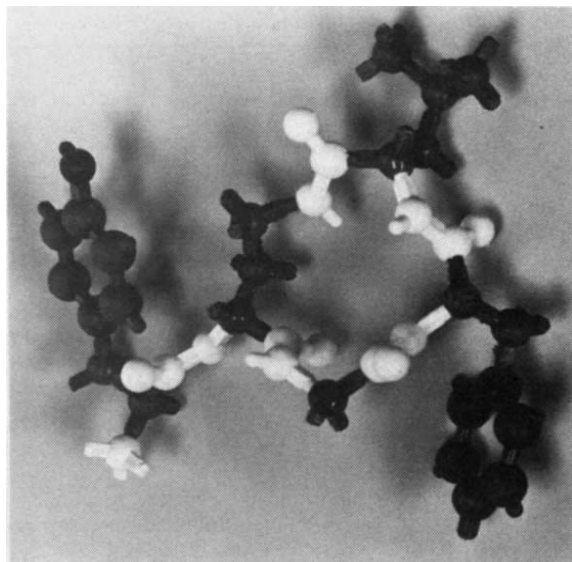
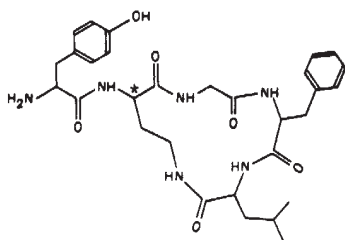


Fig. 4 Molecular model of Tyr-cyclo [-N^γ-D-A₂bu-Gly-Phe-Leu-] constructed from the Leu-enkephalin extended conformation coordinates. A₂bu: diaminobutyric acid. Peptide linkages are shown in white, α-carbons and side chains are dark.

D-amino acids. The only other angle which differs appreciably between enkephalin molecules is ϕ at leucine. These data are compatible with, and may provide an explanation for, the observations that substitution of D-alanine at position 2 and D-leucine at position 5 yield biologically active enkephalin analogues, while substitution of D-phenylalanine at residue 4 destroys activity. Hence, they support the concept of an extended enkephalin conformation as a possible pharmacologically active molecular species.

Determining which of the observed or postulated enkephalin conformations are responsible for binding to receptors and effecting the physiological actions of these compounds would greatly aid our understanding of the nature of opiate-receptor binding and the molecular mechanisms of analgesia. A significant step in this direction has been the synthesis of cyclic Leu-enkephalin analogues with enhanced opiate μ -receptor affinity^{12,13,18}. Substitution of D- α,γ -diaminobutyric acid for glycine in position 2 of the enkephalin sequence and cyclization of the γ -amino group to the leucine C-terminal carboxyl group has produced a compound that is twice as potent as its uncyclized analogue and 20 times as potent as Leu-enkephalin in inhibiting electrically evoked contractions of the guinea pig ileum.



A rationale for this enhanced activity is that cyclization produces a relatively rigid fixed conformation in contrast to Leu-enkephalin which displays both extended and folded shapes in solution and in the solid state, and that this fixed conformation is similar to the 'active' conformation adopted by enkephalins when binding to μ -receptors. It has been demonstrated with molecular models that the cyclized compound cannot retain the

structural features of any of the folded enkephalin conformations; thus, the μ -receptor active conformation almost certainly does not contain an intramolecular bend¹². We have investigated whether our crystal structure extended conformations are compatible with the cyclized enkephalin analogue by constructing a model of the latter compound derived from an extended Leu-enkephalin molecule with the appropriate position 2 substitution and cyclization added. The model is shown in Fig. 4. Analysis of the model reveals that in order to form the cyclic analogue, the linear peptide backbone has to be made somewhat more pleated, with the largest changes in torsion angles occurring at Gly 3. Comparison of Figs 3 and 4 illustrates that although the molecular fit is not exact, the main structural features of the Leu-enkephalin extended conformation can be retained in the cyclized analogue: the peptide backbone is linear; hydrogen-bonding atoms are retained in similar positions and orientations; Tyr and Leu side chains are located on the same side of the backbone, while Phe is on the opposite side. Quantitatively, the Tyr and Phe rings are, respectively, 8.2, 13.3, 13.9 and 8.9 Å apart in the extended molecules A, B, C and D and the same range of distances is permitted in the cyclized model. In terms of arrangement of hydrogen bond acceptors and donors, the distance between Tyr 1 and Gly 3 carbonyl oxygen atoms ranges from 6.8 to 7.2 Å in the extended molecules, while Gly 2 and Phe 4 nitrogen atoms are 6.6–7.1 Å apart. These distances in the cyclized compound are 7.1 and 6.2 Å. It appears, therefore, that if the enhanced activity of the cyclized analogue is due to forcing of the otherwise flexible multi-conformational enkephalin molecule into a conformation similar to that adopted when bound to μ -receptors, then conformations resembling the extended molecules found in this crystal structure are suitable models for μ -receptor active enkephalin stereochemistry.

Apart from the extra atoms introduced by the substitution of diaminobutyric acid for glycine, the biggest change to the enkephalin structure on forming the cyclic analogue is the movement of Leu due to its linkage to the side chain at position 2. It is of interest that for a series of cyclic compounds differing in the identity of the linkage substituent, inhibitory potencies in both the guinea pig ileum bioassay and the rat brain ³H-naloxone binding assay increase with increasing length of the linking side chain¹⁸. As the length of this side chain is increased, we would expect the change in position of the leucine from its location in the extended enkephalin conformation to be lessened in formation of the cyclic compounds. Thus, these biological data are also compatible with and support the concept of an extended enkephalin conformation as the primary candidate for binding to opiate μ -receptors.

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Correlation between luminosity and temperature in γ -ray burst sources

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Observations on Venera 13 and 14, reported here, reveal a strong correlation between the temporal and spectral behaviour of the γ -ray bursts. The instantaneous luminosity of a γ -ray burst is determined primarily by its temperature. This implies that the time profile of the γ -ray bursts directly reflects the spectral variability pattern of radiation. Studying the L versus kT relationship may present a new way of understanding the nature of this puzzling phenomenon.

In the Konus experiment carried out on Venera 11 and 12 during 1978–80, about 150 γ -ray bursts were detected¹. A study of several hundreds of the energy spectra obtained has revealed the spectral variability of radiation to be one of the most characteristic features of γ -ray bursts². It was found that the continuum photon spectra can be approximated by a simple analytical relationship $F \propto E^{-\alpha} \exp(-E/E_0)$. Moreover, it turned out that the parameter α is in most cases close to unity within the experimental accuracy achieved ($\sim 20\%$). The variation of the spectral shape during a burst is governed primarily by variations of the parameter E_0 . It was established that the variation of E_0 usually correlated in time with the burst intensity profile. In many bursts the continua are superimposed by absorption or emission features which have been interpreted as cyclotron and annihilation lines^{2,3}. The variability of the line radiation differs from that of the continuum. The lines, as a rule, are the strongest in the initial phase of a burst.

From a purely formal viewpoint, the observed continuum spectra could originate from the thermal bremsstrahlung radiation of an optically-thin plasma with a temperature $kT = E_0$. However, interpretation of the γ -ray burst spectra as being due to thermal bremsstrahlung radiation meets with serious difficulties², so that the true mechanism of γ -ray burst emission remains unclear. Nevertheless, we may consider the parameter E_0 determining the shape of the spectra as a characteristic of the plasma temperature kT in the γ -ray burst sources.

In the previous observations on Venera 11 and 12 the study of the burst spectral variability was limited by the insufficiently high temporal resolution, ≈ 4 s. In the new observations on Venera 13 and 14⁴ the accumulation time was reduced to 0.5 s for the first two spectra to be measured. This was to improve the accuracy of measuring the spectra of short bursts with a duration < 4 s and of spectral features in the initial phase of longer events. The possibility of a more substantial improvement of time resolution in spectral measurements was limited at the given detector area by the corresponding drop of statistical accuracy. We have, however, succeeded in obtaining new important data on a fast spectral variability of the bursts by comparing the time profiles of a burst recorded with a fast time resolution in two different energy windows. This possibility appeared as a result of a drop of high voltage across the photomultiplier tube in one of the six detectors on Venera 14⁴. The time profile of the γ -ray bursts arriving within the solid angle of maximum sensitivity of this detector is recorded on Venera 14 in a shifted energy window $\Delta E \approx 150$ –700 keV, while on Venera 13 the window was $\Delta E \approx 40$ –180 keV.

The corresponding records of the time profile of the GB820828 and GB820827c events are shown in Fig. 1 with a 0.25-s resolution. The obvious differences in the time profile for two different energy intervals ΔE reflect a spectral variability of the radiation. The hardness of the spectrum in each 0.25-s

interval is characterized by the corresponding count rate ratio N_{14}/N_{13} . The correlation between the spectral hardness and the time profile is so strong that the time behaviour of the ratio N_{14}/N_{13} reflects the finest details in the variation of the radiation intensity. Similar data for the GB820320 event are shown in Fig. 2.

The energy spectra of these events measured in the time intervals specified in Figs 1 and 2 by correspondingly labelled horizontal bars are shown in Fig. 3. These spectra reveal a spectral behaviour which agrees with the previously observed pattern². The radiation temperature falls off during the GB820828 event; an absorption feature is seen to be present in the spectrum in its initial stage. The spectral variability of the GB820827c is more clearly pronounced. During the first second of burst the continuum has an unusual exponential form $F \propto \exp(-E/E_0)$ with $E_0 = 325$ keV. A strong absorption and an emission line are observed. No emission line is seen in subsequent spectra, the absorption line taking more time to disappear. The continuous spectra can now be fitted with the relationship $F \propto E^{-1} \exp(-E/kT)$; the radiation temperature decreases gradually.

The GB820320 event is remarkable not only in the exotic time profile (Fig. 2) but also in its spectrum. Observations of this event from the Solar Maximum Mission (SMM) show that the spectrum, when averaged over the burst, has an extremely hard tail extending as far as $E_\gamma \approx 40$ MeV (ref. 5). The recording of this burst began on Venera 13 and 14 long before the onset of its intensive phase, spectral data being available up to $t - t_0 = 57$ s. Figure 3 shows two spectra of the second narrow peak and a spectrum for the beginning of the most intensive stage. Spectrum 1 reveals an annihilation line, which is no longer present in spectrum 2. A hard tail is seen in spectrum 3 instead of the line. Thus the hard tail in the spectrum of this burst originates from the annihilation feature. According to our data, the same

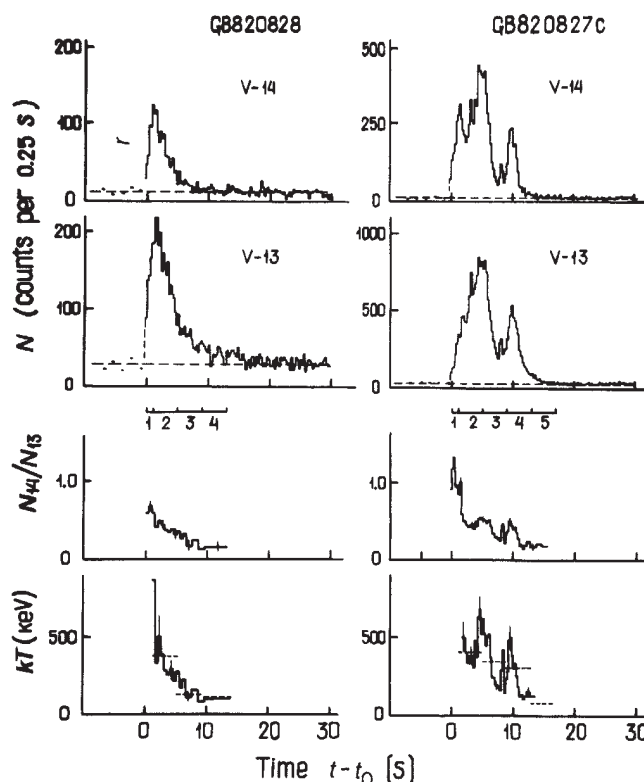


Fig. 1 28 August and 27 August 1982 γ -ray bursts: time profiles (Venera 14 and Venera 13); count rate ratios N_{14}/N_{13} reflecting the instantaneous spectral hardness; temperature kT derived from these ratios. Dashed lines in the lower graphs show mean kT values obtained from spectral measurements in 4-s intervals.

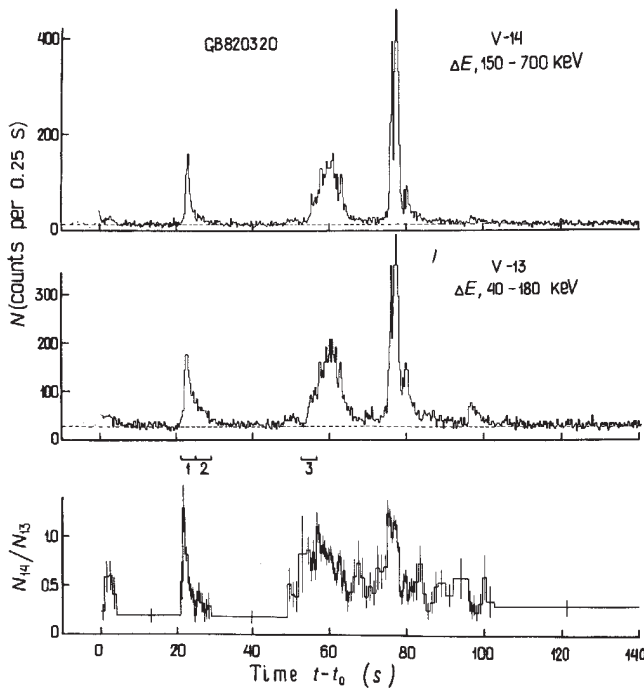


Fig. 2 Time profiles and N_{14}/N_{13} count rate ratios for the 20 March 1982 γ -ray burst.

pattern is also seen in other events whose spectra obtained by SMM⁵ revealed hard tails. This suggests that the plasma in the sources of such bursts is pair dominated.

The N_{14}/N_{13} plots show the real temporal evolution of the energy spectra to be much more complex than would follow from the above averaged spectra. We may assume that in each 0.25-s interval the spectra can be approximated by a $dF \propto E^{-1} \exp(-E/kT) dE$ relationship. This offers then a possibility for evaluating the temperature kT from the magnitude of the N_{14}/N_{13} ratio.

Such an evaluation can obviously only be carried out in cases where the spectrum does not contain strong absorption or emission features. The presence of a feature will result in an overevaluation of the N_{14}/N_{13} ratio and, hence, of kT . Note that the calculated limiting value of the count rate ratio N_{14}/N_{13} for an incident photon spectrum $F(E) \propto E^{-1} \exp(-E/kT)$ with

$kT > 2$ MeV is ≈ 0.7 . The large values of the ratio N_{14}/N_{13} may also characterize strong deviations of spectral shape from the thermal bremsstrahlung when the exponent $\alpha < 1$. Indeed, the peak $N_{14}/N_{13} > 0.7$ in Fig. 1 for the GB820827c seen at $t - t_0 < 1$ s is due not only to the presence of the cyclotron and annihilation lines, but also to an anomalous shape of the continuum (Fig. 3). The large values of the hardness for the GB820320 (Fig. 2) can be attributed to the presence in the spectrum of an annihilation line with a subsequent development of a hard tail.

The values of kT calculated in this way for the $N_{14}/N_{13} < 0.7$ domains in the GB820828 and GB820827c events are presented in Fig. 1. The dashed line shows for comparison mean values of kT derived from the spectra of Fig. 3. We see that when displayed on a fast time scale the correlation between the temperature behaviour and the time profile is very strong. This correlation can be analysed quantitatively. One can use the count rate in the ΔE window and the corresponding magnitude of kT characterizing the overall shape of the spectrum to calculate for each short interval in the time profile the integral energy flux $I(>E)$ in units of $\text{erg cm}^{-2} \text{s}^{-1}$. We have used the values of $N(40-180 \text{ keV})$ as they were measured with a higher statistical accuracy and calculated the magnitude of $I(E > 30 \text{ keV})$ (to compare these values with our spectral data obtained in the energy range 30 keV–2 MeV).

These calculations have led to a surprising result. Figure 4 shows that the instantaneous luminosity $L(>E) = 4\pi d^2 I(>E)$ (where d is distance to the source) and the temperature kT in the source are strongly related by an almost functional relationship, $L \propto (kT)^\gamma$.

A straightforward analysis of the regression curves for these graphs yields for the GB820828 event $L \propto (kT)^{1.70 \pm 0.15}$ with a correlation coefficient $\rho = 0.94$. For the GB820927c event, $\gamma = 1.65 \pm 0.10$, $\rho = 0.93$. In the GB820320, for the weak initial peak, the decaying stage of the second peak and the initial region of the third peak, where $N_{14}/N_{13} < 0.7$, we have obtained $\gamma = 1.0 \pm 0.3$, $\rho = 0.90$; $\gamma = 1.7 \pm 0.3$, $\rho = 0.85$ and $\gamma = 1.5 \pm 0.4$, $\rho = 0.85$, respectively. For the weak event GB821028 also displayed in Fig. 4, $\gamma = 1.0 \pm 0.2$, $\rho = 0.90$.

Note that the most reliable data are those for the first two bursts. The data for the other bursts are based on a small number of experimental points obtained at a poor accuracy of the N_{14} and N_{13} count rate measurements. Therefore they should be considered primarily as supporting the existence of a correlation between the luminosity and temperature.

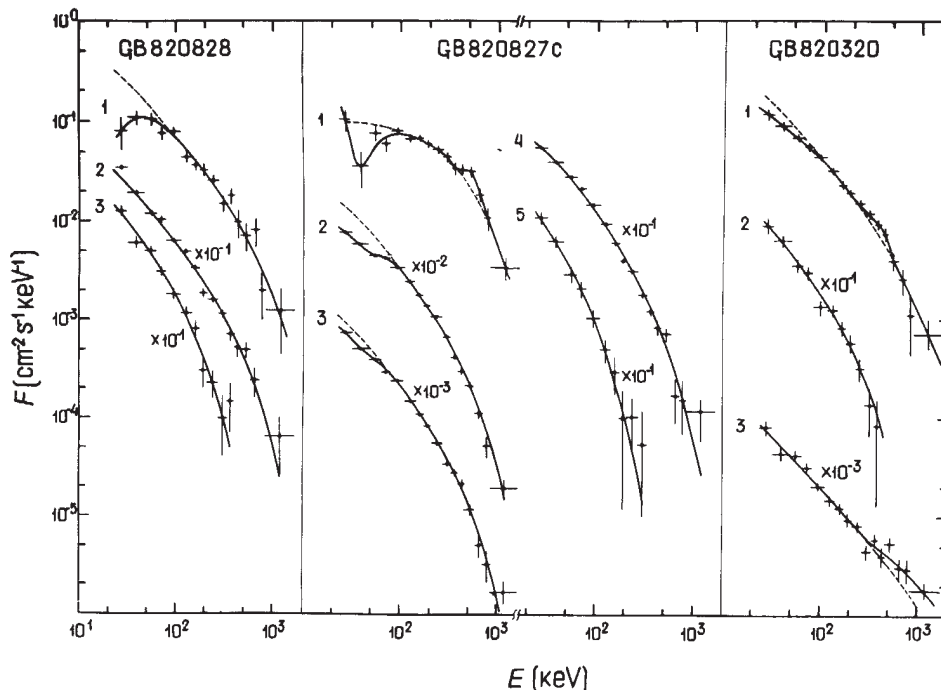


Fig. 3 Energy spectra of the 28 August, 27 August and 20 March 1982 γ -ray bursts in the time intervals labelled accordingly in Figs 1 and 2.

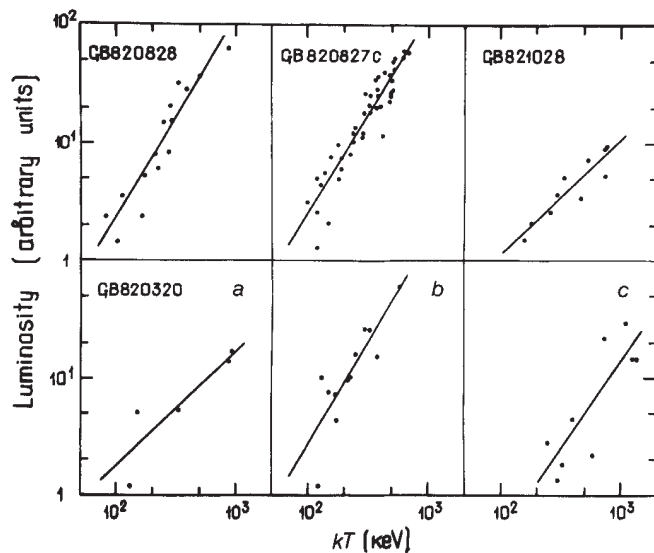


Fig. 4 Instantaneous luminosity versus kT plots. Top GB820828, GB820827c and GB821028 events. Bottom, GB820320 event, for short time profile intervals whose spectra do not contain features: *a*, for the weak initial peak; *b*, for the decaying stage of second peak; *c*, for the initial part of the third peak.

To check the validity of this method, we summed the calculated instantaneous spectra for the GB820828 and GB820827c events for the 4-s intervals of the time profile where such sum spectra were measured directly (Fig. 3). Complete agreement was found within the experimental error between the calculated and directly measured spectra.

Note that the existence of a dependence of luminosity on the source temperature, $L \propto (kT)^\gamma$, finds support when analysing spectral data for several strong γ -ray bursts with structureless, slowly varying time profiles observed on Venera 11 and 12, such as those for GB790419 and GB791101 (ref. 2), where the 4-s time resolution of spectral measurements is sufficient to measure the slowly varying luminosity L and temperature in the sources.

We may conclude that the $L \propto (kT)^\gamma$ relationship holds within a wide range of variation of the temperature kT , 100–1,000 keV, the exponent being $\gamma \approx 1.5$ –1.7. The magnitude of γ can apparently vary somewhat from burst to burst and even for individual rather distant peaks in the same event. This important result implies that for some γ -ray bursts at least, their time profile is directly determined by the spectral variability of the radiation.

Basically, the luminosity of a γ -ray burst source should be a function of plasma temperature and concentration, as well as of the size of the emitting region, $L(T, n, l)$. The clearly-pronounced dependence of luminosity on temperature obtained may imply that the other characteristics of the emitting region remain approximately constant during the burst. One cannot, of course, exclude an alternative possibility that parameters n and l vary in a rigid correlation with temperature. However, this possibility seems to be less plausible.

It is instructive to compare the experimental $L(kT)$ plots with the theoretical relationships derived for various possible emission mechanisms and primarily for the thermal bremsstrahlung of an optically-thin hot plasma. The conventional expression for the thermal bremsstrahlung luminosity of a plasma cloud, $L \propto n_e^2 VT^{1/2}$, disagrees strongly with the observed dependence $L \propto T^\gamma$ with $\gamma \approx 1.6$.

Hot plasma in a strong magnetic field is cooled effectively through thermal synchrotron emission. This process has been suggested as a possible mechanism of γ -ray burst emission^{6–8}. Liang *et al.*⁹ showed that the observed γ -ray burst spectra can be fitted with synchrotron radiation from an optically-thin, mildly relativistic ($kT \ll mc^2$) plasma in a magnetic field B (ref. 10). In this case the shape of the spectrum is determined by a

combination of the parameters, BT^2 . To evaluate B and T separately, one has to invoke additional assumptions^{7,9} which are apparently not the only ones possible¹¹. If we approximate the instantaneous spectra considered here, for instance, for the GB820827c event with the thermal synchrotron radiation, the observed spectral variability will be determined by the variation of the quantity $(B/10^{12})(kT/mc^2)^2$ within 0.1–2, with the instantaneous luminosity varying as $L_{\text{syn}} \propto (BT^2)^{1.5 \pm 0.2}$. Thus in this representation the observed dependence of luminosity on temperature is likewise stronger than predicted, $L_{\text{syn}} \propto n_e VB^2 T^\gamma$, where γ lies within 1–2 (refs 7, 12).

Lamb⁸ has analysed the difficulties encountered when attempting to interpret the γ -ray burst radiation as due to thermal bremsstrahlung, synchrotron mechanism or electron-positron pair annihilation. He pointed out that because of the inherent extremely short plasma cooling time, the energy and/or matter in the γ -ray burst sources should be continuously replenished. Our data may be considered as some evidence that regardless of the mechanism responsible for the γ -ray bursts, it is the flow of energy to the emitting region that governs the spectral variability and, hence, the time profile of the bursts.

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Source of saturnian myriametric radiation

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The Voyager 1 and 2 flybys of Saturn revealed the presence of a variety of plasma waves associated with Saturn's magnetosphere^{1,2}, some electrostatic in nature, others apparently electromagnetic. Possibly the most unambiguous identification of the latter in the low-frequency range (<10 kHz) is of narrow-banded emissions observed over a 3-day period when Voyager 1 was outbound³. Persistent bands of emission near 5 kHz were seen from 19 to 58 R_s (where R_s is Saturn's radius = 60,330 km), and, as the plasma parameters varied widely along the trajectory over this range, it is difficult to envisage how these emissions could be anything but freely-propagating electromagnetic waves. Hitherto their source was unknown, but similar emissions have been observed within the magnetospheres of Jupiter⁴ and Earth^{5–7} and, based on our knowledge of the latter in particular, it is proposed here that the source of the saturnian narrow-band emissions lies in electrostatic upper-hybrid waves near the equatorial plane just beyond the orbit of the moon Rhea.

It is believed that the terrestrial analogue of the saturnian narrowband electromagnetic emissions is terrestrial myriametric radiation (TMR), also known in the past as non-thermal continuum. To standardize the nomenclature, the narrow-band electromagnetic emissions observed at Saturn will be referred to here as saturnian myriametric radiation (SMR), its free space wavelength also being tens of thousands of metres.

TMR is known to derive mainly from electrostatic upper-hybrid waves in the morning sector^{7–9}, which are most intense

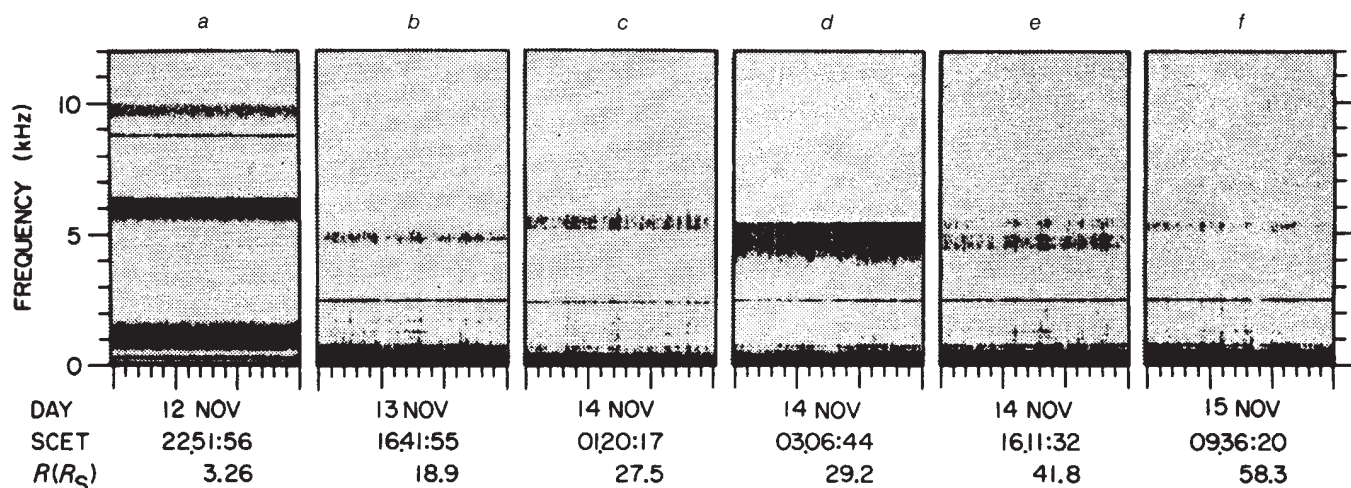


Fig. 1 Frequency-time spectrograms obtained in various regions of Saturn's magnetosphere demonstrating that the emission at ~ 5 kHz can be detected over a large range of radial distances (from ref. 3).

near the geomagnetic equator at the plasmopause and beyond. The upper-hybrid waves derive their energy from the hot electrons drifting around from the night sector. Two theories exist for the generation of TMR from UHR waves, one linear, the other nonlinear.

The linear theory of Jones^{10,11} requires the propagation of the source upper-hybrid waves in a plasma density gradient. As the waves propagate they naturally convert into electromagnetic Z-mode waves¹² which, in certain conditions, can in turn mode-convert through a radio window into left-hand polarized ordinary (L-O) mode waves, the mode in which it is widely believed TMR propagates. The nonlinear theory^{13,14} on the other hand invokes a wave-wave interaction process involving upper-hybrid and low-frequency waves. Barbosa¹⁵ has recently reviewed these theories and concludes the linear theory would achieve a paramount position if the efficiency of the process could be increased. This efficiency problem has been preliminarily addressed elsewhere¹⁶ and will not be considered in detail here. It will suffice to state that the linear process has the necessary efficiency if the wave vectors of the source upper-hybrid waves are not isotropically distributed in azimuth about the magnetic field, but are clustered in the direction of the density gradient. Preliminary measurements by Kurth⁷ appear to confirm that there is an anisotropy in the wave distribution function. Whether this is in the direction required by the linear theory remains to be ascertained.

The linear theory imposes certain restrictions also on the escaping electromagnetic TMR¹⁶⁻²⁰. First, the theory predicts that the TMR will have a frequency equal to the electron plasma frequency f_p at the point of escape, that is at the radio window. It further predicts that TMR will propagate away from the window level into the low-density plasma beyond, with its wave-vector in the plane containing the magnetic field and density gradient vectors at the window. Third, it predicts that within this plane, the wave vector in the magnetospheric cavity will make an angle $\beta = \arctan(f_p/f_c)^{1/2}$ with respect to the magnetic field line at the window, f_c being the electron cyclotron frequency at the window level. It is clear, therefore, that the radiation carries considerable information on plasma conditions at the window through which it has escaped. On this basis, TMR observed by spacecraft in the Earth's magnetospheric cavity has been used to determine certain plasma parameters pertaining to the plasmopause source region¹⁹. It is of considerable interest to apply the same technique to the analogous radiations observed by Voyager 1 (V1) at Saturn in an endeavour to determine where the possible sources could lie and what their plasma characteristics could be. This should allow regions of Saturn's magnetosphere not yet visited directly by spacecraft to be explored.

Figure 1 shows a series of wave spectrograms, obtained by V1 at six positions along its trajectory³. Spectrogram *a* was recorded when V1 was at $3.26 R_S$, in the inner magnetosphere, at local evening. The other frames were all recorded when V1 was outbound from 18.9 to $58.3 R_S$ near 0400 h LT, and it is these which we believe to have derived from the same source region; spectrogram *a* and other wave observations made in Saturn's inner magnetosphere will be considered elsewhere.

Spectrograms *b-f* in Fig. 1 are remarkably similar, bearing in mind that the spacecraft covers a distance during these observations of 2.4×10^6 km. The radiation is seen to be narrow-banded, the lower frequency cutoff in *c* being fairly sharp at ~ 5 kHz, whereas in *d* the upper frequency cutoff at ~ 5 kHz is also rather abrupt. In spectrogram *e*, two bands are visible, one above and one below ~ 5 kHz. It is these signatures of the radiation, together with the remote sensing technique based on the linear theory which will be used to try to determine the source region of the radiations, and its characteristics.

Assuming a dipole magnetic field²¹ of moment $0.21 G R_S^3$ and knowing the spacecraft coordinates at the times that spectrograms *b-f* were recorded, computations according to the linear theory can yield the loci of possible source regions. Figure 2 shows the planet Saturn and, for reference, dipole field lines passing through the orbits of the three moons Tethys (T), Dione (D) and Rhea (R). Also shown is the V1 trajectory projected on to a meridian plane; on the trajectory are marked the positions where the plasma instrument yielded plasma frequencies of approximately 18, 10, 6 and 3 kHz respectively (W. S. Kurth, personal communication). To understand the significance of the contour labelled $\frac{3}{2}f_c = 5$ kHz requires reference to theory and to observations of TMR at Earth.

It is now well established that the main source of TMR lies in electrostatic upper-hybrid waves. The latter are most intense when the upper hybrid resonance frequency $f_{UHR} = (f_p^2 + f_c^2)^{1/2} \approx (n + \frac{1}{2})f_c$ where n is an integer^{8,22,23}. Hence the minimum radial distance at which one expects intense upper-hybrid waves at 5 kHz is where $\frac{3}{2}f_c = 5$ kHz. It is probable, therefore, that the sources of the SMR shown in Fig. 1 do not exist within the contour labelled $\frac{3}{2}f_c = 5$ kHz in Fig. 2.

The curves in Fig. 2 which terminate at the $\frac{3}{2}f_c = 5$ kHz contour are the loci of possible sources of the emissions in Fig. 1*b-f*, according to the linear window theory. These loci were obtained by using as input parameters the centre frequencies of the emissions and the corresponding coordinates of the spacecraft¹⁶. Thus six sets of loci are shown, one each for spectrograms *b*, *c*, *d* and *f* and two for spectrogram *e* which has two frequency bands. It should be added that, for simplicity and in the absence of information to the contrary, the radiation has been assumed to have been emitted in the magnetic meridian plane from the

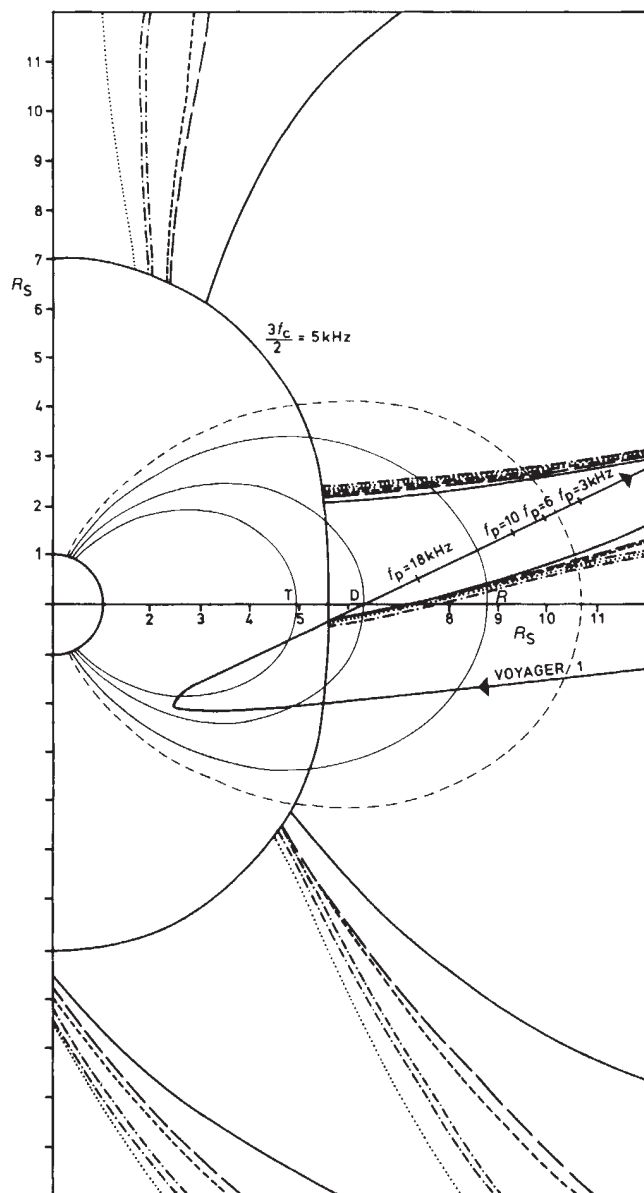


Fig. 2 Meridian plane projection of Voyager 1 trajectory, together with L shells of the moons Tethys (T), Dione (D) and Rhea (R) and $L=10.6$ (dashed). The contour $\frac{3}{2}f_c = 5$ kHz is the inner limit of possible production of electrostatic upper hybrid waves at 5 kHz. The other curves are the loci of possible SMR sources responsible for the emissions in Fig. 1 according to the linear mode-conversion theory. (—b; —c; ----d; -.-e;f). The low-latitude loci are shown in more detail in Fig. 3.

same side of Saturn as lies the spacecraft. Loci of possible sources can easily be computed for other geometrical configurations¹⁶.

The loci in Fig. 2 can be divided into two categories—those at latitudes above 25° and those below this latitude. The higher latitude sources would, for a given radial distance, have to cover a very broad region if they are to be observed by V1 as it moves from 19 to $58 R_S$. This is not the case at the lower latitudes where the loci are so clustered together as to appear as bands and only a relatively small source region is necessary to illuminate the spacecraft along its trajectory. Drawing on experience with terrestrial and jovian emissions where the most intense electrostatic waves lie close to the magnetic equatorial plane^{24,25}, we focus attention here on the loci which lie below latitude 8° .

The V1 plasma instrument recorded a plasma frequency of ~ 5 kHz when outbound at $L \approx 10.6$, and the corresponding magnetic field line is shown dashed in Fig. 2. It is seen to intersect the low-latitude loci $\sim 0.8 R_S$ above the equatorial plane and this region is shown in more detail in Fig. 3b, where the individual

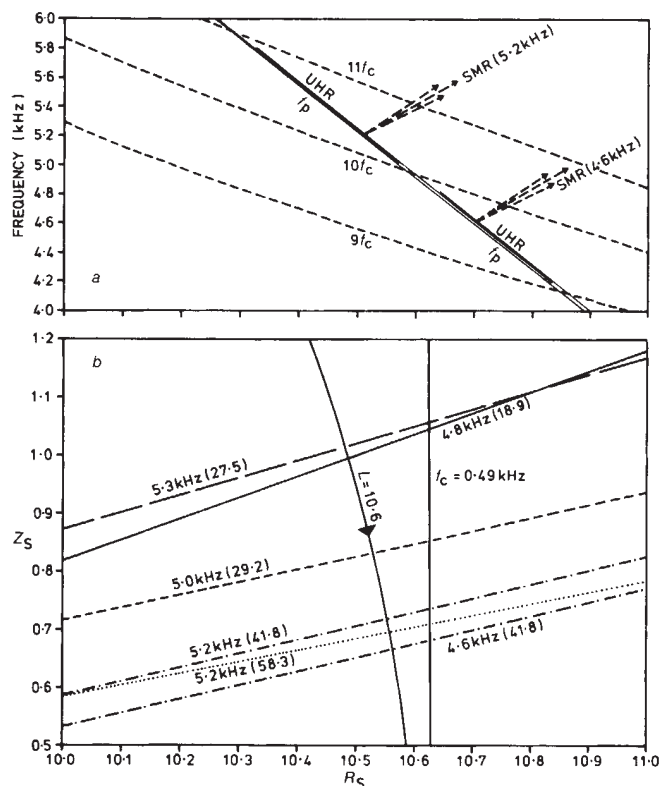


Fig. 3 Detailed diagram (b) of low-latitude loci in range 10–11 R_S . a, An f_p and f_{UHR} profile which could account for the observations in Fig. 1b–f.

loci are now clearly visible. Note that the variation in plasma frequency along the L shell in this region is believed to be small (F. Bagenal, personal communication).

It is proposed that the gap at 4.9–5 kHz in spectrogram *e* in Fig. 2, and the various cutoffs in the other spectrograms, may be taken as evidence of cyclotron damping at the source, and that therefore, on average, $f_{UHR} = nf_c \approx 4.9$ kHz. The value of n which best satisfies a source located near $L=10.6$ in Fig. 3b is $n=10$, thus yielding $f_c = 0.49$ kHz. The contour $f_c = 0.49$ kHz is shown in Fig. 3b.

Figure 3a shows a plasma frequency f_p profile which seems to satisfy the observations. The upper-hybrid frequency closely follows f_p since $f_{UHR} \approx f_p$ when $f_p \gg f_c$. The important points are that when f_{UHR} crosses a cyclotron harmonic, cyclotron damping excludes emission, whereas between harmonics, the instability is expected to intensify^{8,22,23}. The manner in which the SMR is beamed is sketched in Fig. 3a for two frequencies, 4.6 and 5.2 kHz, assuming the abscissa to be parallel to the equatorial plane; the beaming angles with respect to the magnetic field line at the source are 72.6° and 72.1° respectively. The 6-dB beamwidth for a window source can be calculated^{20,26}, and in the present case is found to be 2° . The gradient in f_p which is shown in Fig. 3a is consistent with that observed by V1 when outbound near $L=10.6$ at a latitude of $\sim 10^\circ$.

Note that the source region deduced from these computations lies in the morning sector on the outer flank of the hot plasma torus discovered by V1 particle experiments²⁷. This has similarities to the picture which is emerging from observations of TMR and of jovian kilometric radiation (KOM). TMR sources appear to lie primarily on the plasmopause near the geomagnetic equatorial plane in the morning sector, whereas at least some KOM is believed to have its source on the outer flank of the Io plasma torus, in regions of intense upper hybrid wave activity near the magnetic equatorial plane²⁸.

Gurnett *et al.*³ remark on the evidence of a periodic modulation of the saturnian narrow-band emission intensities at approximately the planet's rotation period (10 h 40 min). The cause of this modulation is unknown, but, on the basis of the

results obtained here, we can speculate on the most probable effects, operating either individually or simultaneously, which could be responsible.

Firstly, the cold plasma parameters in the source region could be modulated such that the process of mode conversion from electrostatic to electromagnetic waves is periodically affected. Within the context of the linear theory, the most critical parameter would appear to be the plasma density gradient. Ray tracing within the Earth's magnetosphere²⁹ indicates that the density gradient needs to be nearly perpendicular to the magnetic field line, thereby allowing the electrostatic waves to have access, through the Z-mode, to the radio window through which the TMR is believed to escape. Second, as the linear theory predicts a beaming of the escaping radiation, it may be that the SMR beams only illuminate the spacecraft at certain times. Given a certain latitudinal width for the source region, a radial variation in its position with SLS longitude would produce a modulation of the beaming angle. For example, an increase in the radial position of the source would, for a given emission frequency, result in the radiation being beamed at smaller angles to the equatorial plane. Therefore a long-lived plasma irregularity on the outer flank of the hot plasma torus, corotating with Saturn could be responsible for the periodic modulation. A similar modulation was seen in observations of narrow band n-KOM at Jupiter, in which the periodicity was slightly less than that of the planet's rotation²⁸. From this it was inferred that the n-KOM derived from a plasma irregularity on the outer flank of the Io torus and, from the difference between the rotation periods, it was inferred that the plasma at Io's orbit was not in strict corotation with Jupiter. Unfortunately, the measurements of SMR would appear to be too few in number for any such conclusion to be drawn concerning the plasma motion at Saturn.

In conclusion, the SMR illustrated in Fig. 1b-f probably derives from a source close to the equatorial plane just beyond $L=10$. It implies the presence of electrostatic upper-hybrid waves and pitch angle diffusion of electrons in a region not visited by the Voyager spacecraft. If there is a local time asymmetry in the production of SMR similar to that for TMR, neither V1 nor V2 would have been expected to observe it when inbound. V1 outbound clearly lies within the beam, albeit only periodically illuminated by it. V2 outbound was at slightly higher latitudes in the southern hemisphere, but at not too different a local time (~0600 h). This seems to confirm that the source, if equally active in both hemispheres, is indeed limited to regions near the equatorial plane and that the radiation is beamed. It should be added that the use of a dipole magnetic field in the computations appears to be well justified for sources near $L=10$, although the additional curvature of the field lines due to a ring current²¹ would result in the sources obtained here being slightly closer to the equatorial plane.

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Anomalous conductivity prefactors in fast ion conductors

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We wish to draw attention to the fact that the conductivity prefactor of arguably the most important fast ion conductor, β'' -alumina, is anomalously large at low temperatures and we show here that this is caused by an unexpectedly high ion hopping rate. In addition, similar results are found in the Na/Ag β -alumina system and these may be of significance in contributing to the understanding of the mixed alkali effect in general.

The conductivities, σ , of many ionic conductors vary with temperature, T , as

$$\sigma = \sigma_0 / T \exp(-E_a/kT) \quad (1)$$

where E_a is an activation energy, k is Boltzmann's constant and the conductivity prefactor σ_0 is given by the expression

$$\sigma_0 = Ne^2 a^2 c (1-c) \gamma k^{-1} \exp(S_a/k) \omega_0 \quad (2)$$

where c is the concentration of mobile ions distributed over N equivalent sites per unit volume, a is the hopping distance, e the electronic charge, γ is a correlation factor, S_a is the entropy of activation and ω_0 the ion vibrational frequency.

At temperatures below ~300 K the conductivity of Na β'' -alumina conforms with equation (1), but is notoriously dependent on sample preparation parameters. A selection of reported σ_0 and E_a values is shown in Table 1. Also, shown for comparison are the widely accepted values for Na β -alumina. It is notable that the conductivity prefactors, σ_0 , of Na β'' -alumina are about two orders of magnitude larger than that of Na β -alumina.

In a recent study of the mixed alkali system Na/Ag β -alumina⁴, conductivity prefactors have been shown to increase with varying Na/Ag compositions despite the system exhibiting a strong mixed alkali effect^{5,6}. The latter effect involves the reduction in conductivity on exchanging Ag^+ for Na^+ ions, and is particularly well developed in this system with conductivities reduced by as many as six orders of magnitude. Such systems, however, may be affected by the weak electrolyte effect⁷, which should act to reduce the concentration of mobile ions. If this is the case, the measured conductivity prefactors must be regarded as anomalously large.

Considerable insight into anomalous prefactors has been obtained by the application of a recently developed⁸ analysis of a.c. conductivity. This analysis is based on Jonscher's 'universal relationship'⁹ for the dielectric loss $\chi''(\omega)$ of hopping ion conductors

$$\chi''(\omega) = \sigma(\omega) / \epsilon_0 \omega \propto (\omega/\omega_p)^{n_1-1} + (\omega/\omega_p)^{n_2-1} \quad (3)$$

in which ω is the angular frequency of measurement, n_1 and n_2 are power law exponents and ω_p is a characteristic frequency. In Na β -alumina, ω_p has been shown to equal internal friction indications of the ion hopping frequency⁸.

Figure 1 shows a.c. conductivity, $\sigma(\omega)$, data for Na β -alumina⁸, Na β'' -alumina¹⁰ and Ag/Na β -alumina¹⁰. Low

Fig. 1 Alternating current conductivity-data for single-crystal Na β -alumina⁸, single crystal Na β'' -alumina¹⁰ and single-crystal Ag_{0.4}Na_{0.6} β -alumina⁴. The characteristic frequencies for the fitted functions are indicated by arrows.

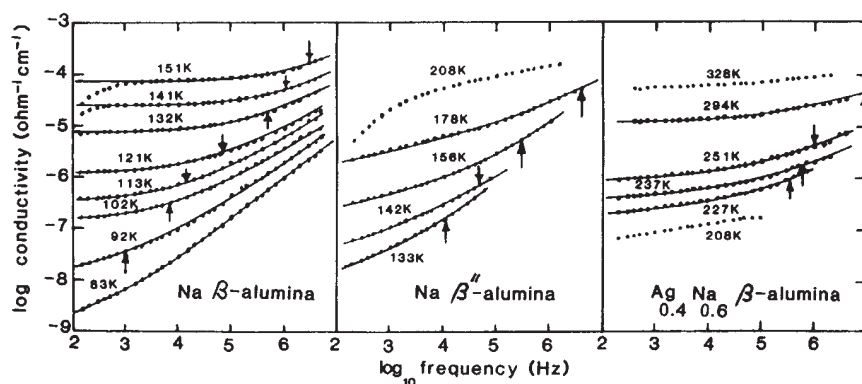


Table 1 Conductivity prefactors, σ_0 , and activation energies, E_a , for Mg stabilized single crystals of Na β'' -alumina, Na β -alumina and the mixed alkali Ag_{0.4}Na_{0.6} β -alumina

Preparation temperature (°C)	σ_0 ($\Omega^{-1} \text{ cm}^{-1} \text{ K}$)	E_a (eV)
1,660 (ref. 1)	2×10^5	0.22
1,690–1,730 (ref. 1)	8×10^5	0.31–0.36
1,650 (ref. 1)	8×10^5	0.26
1,700 (ref. 2)	6×10^6	0.33
1,700 (this work)	1×10^5	0.26
Na β -alumina (ref. 3)	2.37×10^3	0.165
Ag _{0.4} Na _{0.6} β -alumina (this work, ref. 4)	7×10^3	0.35

Table 2 Estimates of the carrier concentration factor for the three electrolytes

	Na β -alumina	Na β'' -alumina	Ag _{0.4} Na _{0.6} β -alumina
$\sigma T / \omega_p$ ($\Omega^{-1} \text{ cm}^{-1} \text{ Hz}^{-1} \text{ K}$)	8×10^{-10}	2×10^{-10}	8×10^{-11}

frequency dispersion¹¹ was significant in both Na β'' -alumina and Ag_{0.4}Na_{0.6} β -alumina and was accounted for by modifying the previous technique⁸ for the extraction of ω_p from a.c. data. These modifications amount to using the general form of the a.c. conductivity expressed in equation (3) and will be discussed elsewhere. Arrhenius plots of derived characteristic frequencies, ω_p , and d.c. conductivities are shown in Fig. 2. Despite the small number of data sets that could be fully analysed in this way, it is evident that the ω_p values of both Na β'' -alumina and Ag_{0.4}Na_{0.6} β -alumina are anomalously high. The effective attempt frequencies given by the infinite temperature intercept, are much larger than the value 3×10^{12} Hz, for Na- β alumina. These values, equal to $\exp(S_a/k)\omega_0$ in equation (2), appear to be the cause of the anomalously high conductivity prefactors for these materials. An indication of the relative values of mobile ion concentration in the three conductors is obtained by comparing the ratios $T\sigma/\omega_p$. Values of this quantity are shown in Table 2. The difference between Na β and Na β'' -alumina can be largely explained by the shorter hopping distance, appearing as a^2 in equation (2), in Na β'' -alumina. Hence we conclude that, at these low temperatures, the mobile ion concentration in Na β'' -alumina is probably similar to the value of about 20% obtained for Na β -alumina⁸. In the mixed alkali system, however, the concentration appears to be an order of magnitude lower, providing evidence of the weak electrolyte effect. In this case, the expected reduction in the conductivity prefactor is balanced by the anomalously high values of ω_p .

Nuclear magnetic resonance studies¹² of Na β'' -alumina and internal friction studies¹³ of another mixed alkali system also gave indications of unusually high hopping rates. Their physical origin, however, is not at all clear. The high effective attempt frequency can be parameterized by introducing an entropy

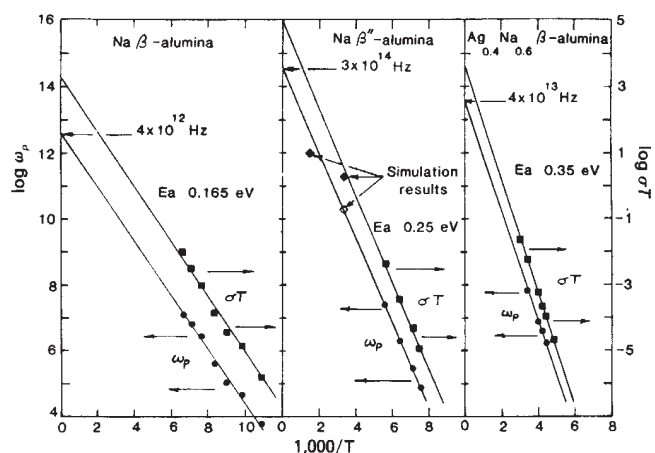


Fig. 2 Arrhenius plots of conductivity, σT , and characteristic frequency ω_p for Na β -alumina, Na β'' -alumina and Ag_{0.4}Na_{0.6} β -alumina. Results from a molecular dynamics simulation of Na β'' -alumina¹⁰ ($\diamond$) are included for comparison with the experimental estimates of ω_p . The 300 K result is also shown, $\diamond$, reduced by a factor of 10 to account for the error introduced by ordering effects¹⁴.

activation. How this or other factors which govern ion hopping rates should be interpreted in high ion density, strongly interactive ionic conductors needs considerable clarification. It is hoped that the advent of realistic molecular dynamics simulations of these materials will answer some of these questions. Two estimates of inverse mean residence times from such a simulation¹⁴ of Na β'' -alumina are shown in Fig. 2. The 300 K result was found to overestimate the conductivity by about an order of magnitude because the simulation failed to cope with ordering effects. If it is reduced by an order of magnitude the agreement with the extrapolation of our results is excellent. It is hoped that successful comparisons of this kind may finally elucidate the origins of the characteristic times obtained by the various experimental techniques.

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Recent temperature changes in the Arctic and Antarctic

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The possibility of a global warming induced by increasing levels of atmospheric carbon dioxide has led to increased interest in monitoring global temperatures. Polar regions are of particular interest because temperature changes may be amplified in these regions by the complex feedback mechanisms which operate in the atmosphere-ice-ocean system¹. Monthly mean station surface air-temperature data have been objectively analysed to produce time series of temperature anomalies for the Northern Hemisphere (1881–1980)^{2,3}, the Arctic (1881–1980)⁴ and the Antarctic (1957–82)⁵. A comparison of recent changes in these three regions is made here. Previously published series have been updated to 1982. Changes in Arctic temperatures since about 1966 and in Antarctic temperatures since 1960 show significant warming trends. For the period from 1975 to 1982 there is a high positive correlation between temperature variations in the Arctic and Antarctic.

To minimize the effect of differing altitude and aspect, temperatures were calculated as anomalies from a reference period with good data coverage. For the Northern Hemisphere the reference period 1946–60 was used and for the Antarctic it was 1957–75. Before 1957 there were not enough recording stations in the Antarctic to be able to calculate a meaningful area average. Even then, there were only 16 stations available for the Antarctic compared with about 60 for the Arctic. The station anomaly data were interpolated onto a 5° latitude by 10° longitude grid using an inverse-distance-weighted best fit plane². The number of grid points for which values could be interpolated since 1957 averaged ~60% for the Northern Hemisphere as a whole, 80% for the Arctic (65–90° N) and 50% for the Antarctic (65–90° S). Areal averages were formed using latitudinally-weighted grid point values. These data are mostly representative of conditions over the landmasses.

Time series of annual averages are shown in Fig. 1, with superimposed smoothed values calculated using a 9-weight binomial filter. Seasonal values for the Arctic and Antarctic are shown in Fig. 2. We note that for the period 1957–82 the variances of all the Antarctic seasonal estimates, except spring, are significantly greater (5% level) than the equivalent season in the Arctic. Whether this is a real climatic difference, or simply the result of differing data density and/or the differences in data coverage is uncertain. There is no statistically significant difference between the variances of the Arctic and Antarctic annual series for 1957–82. We restrict further discussion to the annual data.

As part of a longer term cooling trend which began about 1940, temperatures in the Arctic decreased from the mid-1950s to the mid-1960s. The mid-1960s mark the turning point between this cooling trend and a subsequent warming. The temperature anomaly in 1966 was the coolest since 1919. From the mid-1960s until 1982 the Arctic series has a warming trend, the linear component of which ($0.035^{\circ}\text{C yr}^{-1}$) accounts for 18% of the variance. During this period, a warming trend, of smaller magnitude, can also be seen in the Northern Hemisphere series. For this larger region there were notably large year-to-year variations (especially since 1970) so the warming trend, from the mid-1960s until 1982, accounts for only a small fraction of the variance. For the Antarctic, temperatures were coolest in 1960. During the 1960s and the first half of the 1970s there

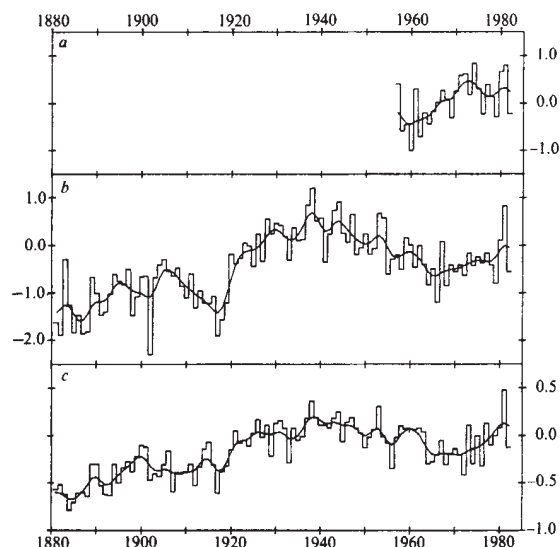


Fig. 1 Annual temperature anomalies ($^{\circ}\text{C}$) averaged over *a*, the Antarctic (65–90° S) with reference period 1957–75; *b*, the Arctic (65–90° N) with reference period 1946–60; and *c*, the Northern Hemisphere (0–90° N) with reference period as in *b*. Superimposed smoothed values were calculated using a 9-weight binomial filter. The end points were estimated by assuming past/future values similar to the averages of the first/last 5 yr. (Note change of scale for the Northern Hemisphere.)

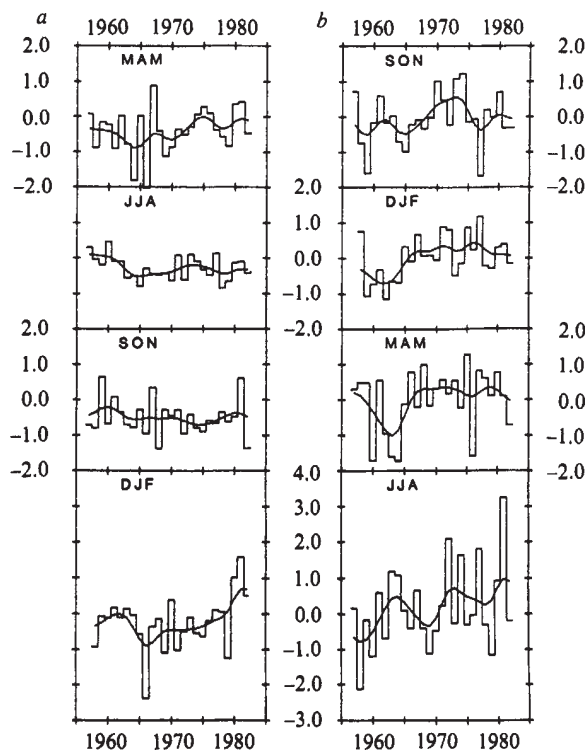


Fig. 2 Seasonal temperature anomalies ($^{\circ}\text{C}$) averaged over *a*, the Arctic (65–90° N); and *b*, the Antarctic (65–90° S). Reference periods and smoothed values as in Fig. 1. Seasons are identified by the months (spring for the Northern Hemisphere is MAM and for the Southern Hemisphere is SON).

was rapid warming; the linear trend from 1960 to 1974 amounts to $0.085^{\circ}\text{C yr}^{-1}$ and accounts for 57% of the variance. In the second half of the 1970s and the early 1980s, large year-to-year variability obscures any clear trend. In both polar regions, the recent warming has been most notable in winter (Fig. 2).

We now consider whether there is any relationship between the temperature variations in the two polar regions. When the whole data period is considered, there is no significant correlation between the Arctic and Antarctic annual series at zero-lag

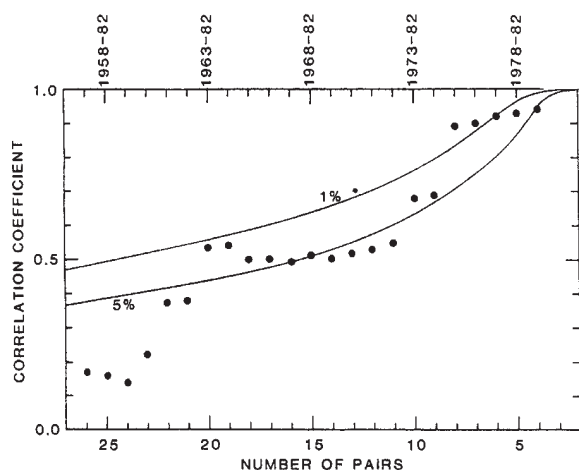


Fig. 3 Correlation coefficients between annual Arctic and Antarctic temperatures for a diminishing number of years from 1957-82 ($n=26$) to 1979-82 ($n=4$). The curves show the correlation coefficients required to reach the 5% and 1% significance levels, but the implied levels can only be taken as a rough guide and must overestimate the true significance because of the multiplicity of tests performed.

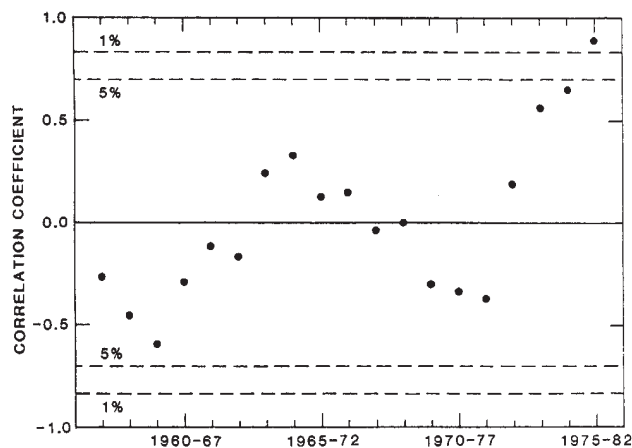


Fig. 4 Correlation coefficients between annual Arctic and Antarctic temperatures for 8-yr periods from 1957-64 to 1975-82. The high positive correlations in recent years are not as significant as indicated because of the multiplicity of tests carried out, but precise significance levels cannot be calculated because the individual correlations are not independent.

($r=0.17$). When the Arctic is lagged 6 yr behind the Antarctic, so bringing in line the turning points between trends in the two series, the correlation coefficient is 0.65. This result, which is significant at the 1% level after allowing for autocorrelation, largely reflects the similar, albeit lagged, cooling and warming trends in the two records. The fact that the probability of getting a high correlation is increased when a series of correlations is computed has not been taken into account in the significance test. It would therefore be unwise to place undue weight on this result in the absence of a theoretical causal mechanism which might explain a lag of ~6 yr between the polar regions.

Although there is no significant correlation between the temperature changes in the Arctic and Antarctic at zero-lag for the whole period 1957-82, the changes which have occurred in the most recent years are similar in both regions. In particular, we note the sequences for 1979 through 1982: cool, warm and warmer for the first three years, followed by a large drop in 1982. (The warmth of 1981 in all records is remarkable. In Antarctica this is largely the result of an exceptionally warm winter (Fig. 2) and further analysis of this event is required.) Figures 3 and 4 show the results of correlations calculated to test the strength and duration of the recent similarity in Arctic and Antarctic temperature variations. It is clear that, in general,

Arctic and Antarctic temperature variations are not related. Any explanation for the high correlation between the temperature changes in the two polar regions since 1975 most probably lies in the internal complexities of the global climate system rather than in external climate forcing. The relationship, while strong at present, is likely to be transitory.

It is, however, probable that these three temperature records do reflect changes in external forcing, but the mechanisms involved are as yet too poorly understood for us to make any positive identifications. The primary candidates for external climate forcing are volcanic activity, changes in solar output and carbon dioxide^{6,7}. A possible contributing factor to the relative coolness of 1982 in the Arctic and Northern Hemisphere as a whole may be the stratospheric dust and aerosol loading caused by the eruption of the Mexican volcano El Chichón^{8,9}. It is unlikely, however, that the coolness of 1982 in the Antarctic was a direct result of this eruption. Although the recent decadal time scale warming shown in all three records seems to be consistent with the hypothesized effects of increasing carbon dioxide concentration on climate^{6,10}, the duration and magnitude of the warming are insufficient to provide independent empirical verification of the greenhouse effect¹¹. Continued monitoring of climatic parameters on a global basis is essential for the early detection of large-scale climatic change.

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Noril'sk only a minor contributor to Arctic haze

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The Arctic atmosphere contains considerable pollution aerosol during winter¹⁻⁴. Although the main source areas of this material, known commonly as 'Arctic haze', are not yet known with certainty, chemical and meteorological evidence points increasingly to Eurasia^{5,6}. Aerosol of the North American Arctic seems to come mostly from the central Soviet Union, while aerosol of the Norwegian Arctic comes primarily from Europe and the western Soviet Union^{2,5-8}. Interestingly, pollution sources within the Arctic seem to be generally unimportant regionally. One possible exception is the copper/nickel-smelting complex at Noril'sk, in the Soviet Union (69.4° N, 88.5° E), which is a strong, isolated source near one of the major atmospheric pathways to the Arctic⁸. The nearest strong sources upwind along this pathway lie at roughly 55° N and 70° E, or 1,500 km south and west. On the basis of its sulphur dioxide emissions, Noril'sk has been suggested to be a major source of Arctic sulphate aerosol⁹. Using trace elements, we have recently detected the Noril'sk plume at Barrow, Alaska, and now use the resulting data to show that Noril'sk is not a major contributor to Arctic sulphate.

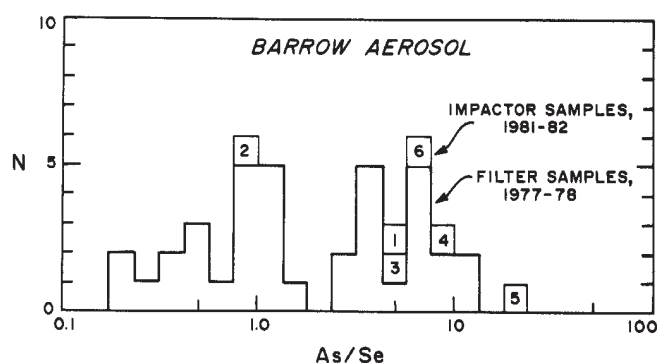


Fig. 1 As/Se frequency distribution for the Barrow aerosol of winter.

The Noril'sk signal appeared in one of six high-volume cascade impactor samples taken at the surface in Barrow between December 1981 and March 1982. The samples were analysed for 40 elements by neutron activation and for sulphate by turbidimetry. Selected total elemental concentrations and ratios to selenium (for ease in comparing with other samples) from the impactor samples are shown in Table 1, together with comparative data from 21 filter samples taken at the same site during December 1977–March 1978.

Most concentrations were low in the first three impactor samples (whose air masses had not come directly from any strongly polluted areas), but were much higher in the last three samples (which were associated with more direct flow from the Soviet Union). Ratios to Se were more similar in the two sets, but for some elements were higher in the second set. Concentrations in the impactor samples were generally within the means from the filter samples of 1977–78.

Impactor sample 5 was anomalous. Its arsenic/selenium ratio of 18 was well above those of the other impactors and was 4 s.d. above the mean of the filter samples. Figure 1 is the As/Se frequency distribution for all samples analysed to date from Barrow, and shows clearly how the value for sample 5 fell outside the range of the other 42 samples. To a lesser extent, sample 5 was also enriched in indium, antimony and zinc.

The pattern of enrichments in impactor 5 is characteristic of smelters^{10,11}. For example, we have recently used In to detect aerosol from Canadian Cu/Ni smelters after transport of

1,000 km to Rhode Island¹². The In/Se ratio of sample 5 was near the mean from the Canadian smelters, which use ores similar to those of Noril'sk, and was considerably above values in coal-burning or oil-burning areas. (We were not able to use Cu or Ni as tracers here—each had large analytical uncertainties, and Ni was masked by the anodizing finish on the impactors.) The lack of significant enrichment for elements such as iron, chromium, noncrustal manganese and noncrustal vanadium in sample 5 was also consistent with previous direct studies of smelter effluent^{10,11}.

Strong evidence that Noril'sk was responsible for the smelter signal in sample 5 came from meteorology and from chemical measurements much closer to the source. We have deduced the general air-flow patterns from the surface and 500-mbar meteorological charts for the dates preceding and including those of the samples of interest. The early part of sample 5 was marked by a zone of strong northeastward flow of air from the Noril'sk area to the Beaufort Sea, a synoptic situation which occurs only infrequently. The air of samples 4 and 6 had similarly strong northward flow on the Eurasian side, but originated farther south in Eurasia and entered the Arctic in broader zones centred east and west of Noril'sk, respectively. Finally, our tracer-element ratios for sample 5 essentially matched those of the Noril'sk plume, as determined from aircraft samples taken at altitudes of 750–1,500 m (925–850 mbar) over the Arctic Ocean, about 1,500 km north-east of Noril'sk. Of a set of filter samples taken during winter 1980–81, five, during January 1981, were found to have a distinct and reproducible smelter-like signature, which is given in the last column of Table 1. Each of these five samples was taken in air which had passed eastward over the Noril'sk area and then curved northward over the Arctic Ocean. The smelter characteristics of impactor sample 5, the extreme similarity of its elemental ratios to those measured in the Noril'sk plume aloft, and the meteorology of sample 5 have convinced us that we have indeed identified the Noril'sk aerosol after transport of 4,000 km to Barrow. (Influence of Noril'sk could also explain unusual maxima of Zn observed previously at Mould Bay, N.W. Territories, Canada, during November 1979 and February 1980², in association with air flow similar to that of our sample 5. Unfortunately, data on As, Sb, Se and In are not available to verify the Noril'sk signature.)

The Noril'sk plume brought only small amounts of sulphate to Barrow. Table 1 shows that the sulphate/Se ratio was 22–27 ×

Table 1 Selected elemental concentrations (ng m⁻³) and ratios in Arctic aerosol

	Filter samples Dec. 1977– Mar. 1978 (n = 21)	Barrow, Alaska, surface Cascade-impactor samples, December 1981–March 1982						79–85° N, 100–150° E alt. 750–1,500 m Filter samples, January 1981 (n = 5)
		1 12–19 Dec.	2 21–26 Dec.	3 26 Dec.– 3 Jan.	4 10–22 Jan.	5 2–9 Mar.	6 11–23 Mar.	
As	0.61 ± 0.42	0.17	0.029	0.148	1.22	3.2	0.83	4.7 ± 3.7
In	0.0036 ± 0.0040	—	0.00130	0.00088	0.00275	0.0106	0.0046	0.0114 ± 0.0073
Sb	0.125 ± 0.079	0.044	0.033	0.044	0.022	0.38	0.168	0.53 ± 0.36
Zn	5.3 ± 2.7	1.49	1.82	2.5	10.0	15.7	7.9	24 ± 13
Se	0.109 ± 0.045	0.032	0.037	0.035	0.143	0.172	0.142	0.177 ± 0.111
Noncrustal Mn	0.71 ± 0.44	0.79	0.45	0.44	1.32	2.7	1.47	2.3 ± 0.9
Cr	0.37 ± 0.20	0.49	0.49	0.37	1.07	2.8	1.03	—
Noncrustal V	0.73 ± 0.62	0.20	0.189	0.167	1.48	2.5	1.54	1.62 ± 0.84
Fe	22 ± 9	9.8	14.2	15.3	103	76	38	—
Sc	0.0046 ± 0.0022	0.00160	0.00152	0.0027	0.026	0.0146	0.0065	0.030 ± 0.023
SO ₄ ²⁻	1300 ± 530	850	810	920	1580	3900	3600	—
As/Se	5.2 ± 2.9	5.4	0.78	4.3	8.5	18.6	5.9	24 ± 6
In/Se	0.030 ± 0.020	—	0.036	0.025	0.019	0.062	0.032	0.061 ± 0.018
Sb/Se	1.18 ± 0.54	1.35	0.91	1.27	1.52	2.2	1.18	2.8 ± 0.4
Zn/Se	50 ± 20	46	50	72	70	91	56	139 ± 20
Noncrustal Mn/Se	6.3 ± 3.1	12	11	13	9	16	10	14.9 ± 4.9
Cr/Se	3.5 ± 1.1	15	13	11	7.5	16	7.3	—
Noncrustal V/Se	5.4 ± 3.8	6.2	5.0	4.8	10.3	14.7	10.8	9.7 ± 1.4
Fe/Se	220 ± 60	310	390	440	720	440	260	—
Sc/Se	0.046 ± 0.021	0.050	0.042	0.077	0.183	0.085	0.046	0.185 ± 0.108
SO ₄ ²⁻ /Se (× 10 ⁻³)	11.9 ± 6.9	27	22	26	11.0	23	25	—

10^3 in five of the six impactor samples, including sample 5. In other words, sample 5 had no detectable enrichment of sulphate relative to Se. A similar observation can be made relative to Fe, scandium and noncrustal V. Considering the analytical uncertainties for sulphate and the tracer elements (generally 5–20%), we have concluded that Noril'sk cannot have contributed more than about 10–25% of the sulphate to sample 5, that is, less than about $0.4\text{--}1\text{ }\mu\text{g m}^{-3}$. When the Noril'sk signature is not detected, its contribution to Arctic sulphate is probably less than 10% of the total, or less than $0.2\text{--}0.3\text{ }\mu\text{g m}^{-3}$.

This small contribution to sulphate is reasonable on several grounds: (1) A point source, no matter how strong, is unlikely to be responsible for the high concentrations of sulphate and its consistent proportions to the rest of the aerosol such as are observed throughout the Arctic during winter and spring. (2) It is in proportion to the order-of-magnitude higher SO_2 emissions in the industrialized southern Urals and vicinity, which is the area generally referred to as the 'central Soviet Union'. (3) Noril'sk is not needed to account for Arctic sulphate—concentrations similar to those at Barrow are also found in the Norwegian Arctic, whose aerosol comes from Europe and the western Soviet Union. (4) We have recently observed that the plumes from the large Cu/Ni smelters in eastern Canada

contain less than $1\text{--}1.5\text{ }\mu\text{g m}^{-3}$ sulphate aerosol after transport to Rhode Island, or about 10 times less than predicted if all their SO_2 had been converted¹². The earlier assumed total conversion of SO_2 in the Noril'sk plume, which led to an estimated $4\text{ }\mu\text{g m}^{-3}$ sulphate at Barrow⁹, was thus probably much too high. Furthermore, the elements used there as indicators for Noril'sk (titanium, Cr, Mn, Fe, Ni) are not tracers of smelters; they cannot even be considered tracers of a given area unless their proportions are specified precisely.

In conclusion, it appears that the Noril'sk smelting complex can indeed be detected at Barrow, but only rarely. Its signal may be absent or masked for an entire winter at a time. Even when the Noril'sk plume is detectable, it affects only a few elements and brings only small additional sulphate. Thus, Arctic haze still seems to be attributable to regional pollution sources in mid-latitudes.

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Magnetic fabric investigations of pyroclastic deposits from Phlegrean Fields, southern Italy

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Flow regimes, depositional conditions and location of the sources for pyroclastic flows are difficult to study by conventional methods^{1,2}. Magnetic fabric techniques have the advantage that they can be undertaken rapidly and precisely, the measurement of a single sample taking only a few minutes³. This study was of samples collected from the Quaternary Fiumicello Formation of surge deposits in the Phlegrean Fields near Naples. These samples had originally been collected for standard palaeomagnetic studies, but it was decided to investigate their magnetic fabric as the areas had been recently studied geologically with the objective of determining their source⁴. The magnetic parameters in the pyroclastic surge deposit were found to be identical to those found in undeformed sediments and we conclude that the controlling conditions must be similar. This suggests that magnetic fabrics may be used as a fast, first-order method of determining both the source area for such flows and their conditions of formation.

The volcanic activity in the Phlegrean Fields developed during a Plio-Quaternary extensional phase associated with the deepening of the sedimentary basement along a series of NW–SE, NE–SW and E–W faults. The undersaturated potassic volcanism evolved in composition from basaltic to trachytic, with explosive

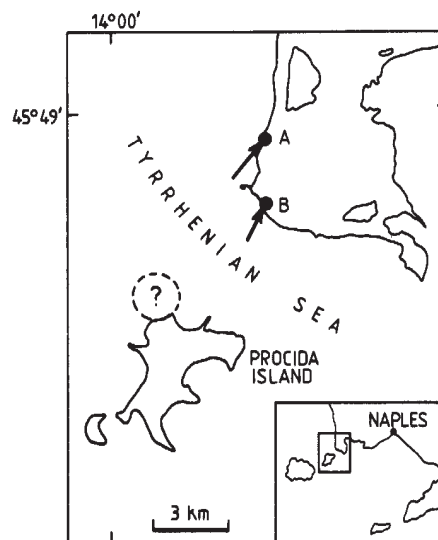


Fig. 1 Location of the Torregaveta (A) and Acquamorta (B) outcrops. The arrow indicates palaeoflow directions as indicated by the magnetic fabric. The suspected source, on geological grounds⁴, is near Procida, indicated by the dashed circle.

phenomena prevailing during the late stages of activity^{5–7}. The trachybasaltic surge sequence outcropping at Torregaveta and Acquamorta (A and B respectively in Fig. 1) represents the distal (planar) facies of a surge phenomenon originating⁴ from the Fiumicello volcano (dashed circle, Fig. 1), as evidenced by its petrology, ¹⁴C dating, facies, petrochemical and grain size analyses. The Fiumicello Formation consists of alternations of a few centimetres of dark ashes, intercalated with tens of centimetres of thick black scoriae and lapilli strata. The total thickness at Torregaveta is about 2 m and nearly 8 m at Acquamorta. Five different ash strata were sampled, with 25 drill cores from Acquamorta and 30 from Torregaveta. At two sites (A2 and B2), the samples were from both the upper and lower levels of the strata. Site A1 (Torregaveta) was from the actual base in

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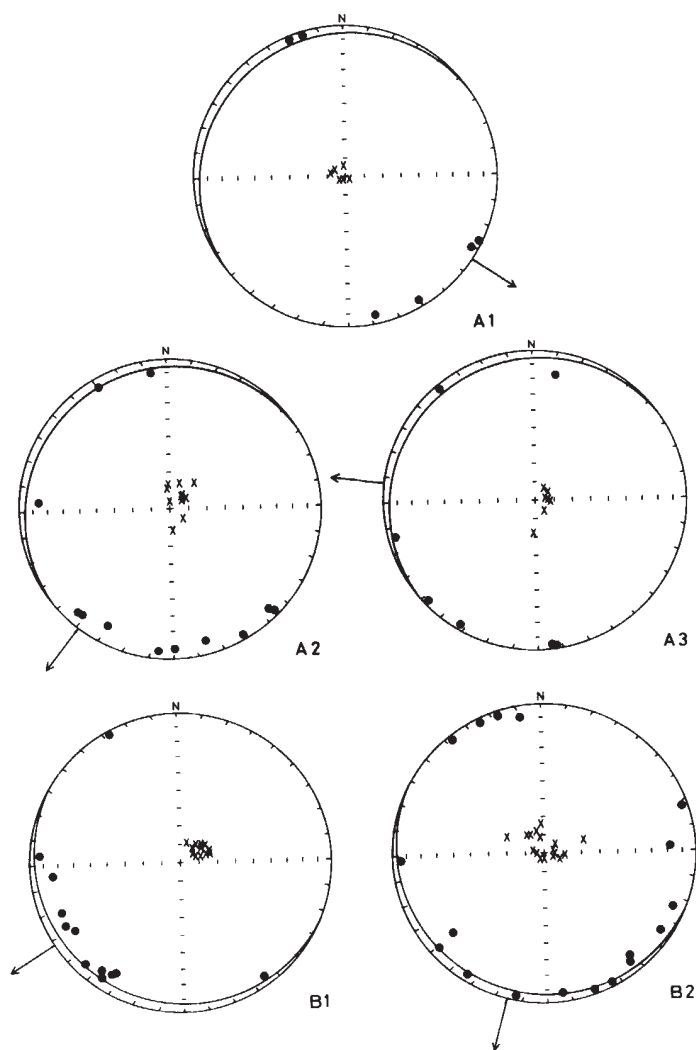


Fig. 2 Equal-area lower hemisphere stereographic projections of maximum (●) and minimum (×) susceptibility axes. Locations A and B as in Fig. 1. Arrows indicate the mean direction of dip of the magnetic foliation planes based on the imbrication defined by the tilt of the mean minimum susceptibility axes. The data are uncorrected for the shallow 'bedding', which is shown as a large circle, as such recent strata have not tilted after their deposition and this plane therefore represents the palaeodepositional surface, except in the case of A1.

which the lower surface closely matches the small-scale unevenness of the depositional surface, and B1 (Acquamorta) was sampled from the base of the same flow. All measurements of magnetic fabric were with a modified Digico anisotropy delineator⁸ and all sites were found to have similar bulk susceptibilities (Table 1). Studies of the magnetic minerals⁹ showed that magnetite was the carrier of the magnetization in all sites.

There were no fundamental differences in the magnetic fabric in the upper and lower levels (sites A2 and B2) indicating that the fabric is consistent within each ash layer. In all cases, the three axes of the magnetic susceptibility ellipsoids show similar features to those found in undeformed sediments¹⁰ (Table 1), the maximum and intermediate axes being intermingled within a girdle close to 'bedding', that is the palaeodepositional surface, and the minimum axes oriented close to vertical (Fig. 2). This similarity with unconsolidated sediments is further emphasized by other magnetic parameters, such as q (ref. 11), and suggests that the forces operating during deposition of the ashes were similar. As occurs in undeformed sediments, the directions of the maximum and intermediate (not illustrated) susceptibility axes are largely intermixed so that their mean directions are not orthogonal. This fact also restricts any definition of a lineation using only the maximum susceptibility axes. However,

Table 1 Mean site parameters

Site	No. of cores	$K_{\max}$ Decl.	$K_{\max}$ Incl.	$K_{\min}$ Decl.	$K_{\min}$ Incl.	α_{95}	K_{mean} ($4\pi \times 10^{-3}$)	q
A1	6	147	2	303	87	4	2.40 ± 0.17	0.08 ± 0.02
A2	12	177	3	39	83	5	2.00 ± 0.10	0.29 ± 0.18
A3	7	196	2	99	85	6	1.99 ± 0.14	0.17 ± 0.05
B1	12	250	16	59	76	3	2.31 ± 0.09	0.11 ± 0.05
B2	18	168	26	17	85	5	1.49 ± 0.12	0.33 ± 0.22

The mean directions of the $K_{\max}$ axes are given without statistical parameters as both $K_{\max}$ and $K_{\min}$ lie within a girdle and the low q values indicate that the individual ellipsoids are strongly oblate so that the precise directions of either axis are meaningless. For this reason, the mean $K_{\min}$ value is also omitted. The $K_{\min}$ directions are well defined and are given with their cone of confidence. Average K_{mean} ($(K_{\max} + K_{\text{int}} + K_{\min})/3$) and average q values, with associated standard deviations, are given assuming a normal distribution of these parameters.

the tilt of this plane, its imbrication, was quite well defined by the tilt of the minimum susceptibility axes (Fig. 2). The imbrication of the magnetic fabric at sites A2, B1 and B2 (Fig. 2) is consistent with their having been derived from a source to the south-west, at Procida, exactly as indicated on geological grounds⁴ (Fig. 1), but the imbrication at site A3 appears significantly different (Fig. 2) and site A1 has an apparently negative imbrication. The q parameters, widely used in sedimentological studies^{9,12}, all fall in the range 0.08–0.33 (Table 1). The lower values being consistent with those obtained for sands deposited on a sloping bed in stationary water¹², and the higher values comparable with those for sand deposited in flowing^{13,14} water.

The apparent identity between the magnetic parameters in these pyroclastic deposits and those of normal subaqueous sediments prompts similar analysis of the modes of deposition in the different pyroclastic sites. Sites A1, A3 and B1 have low values of q , suggesting that the stresses due to flow over a sloping surface were lower than the tangential gravitational stresses. The negative magnetic imbrication at site A1 would appear inconsistent with such an interpretation. However, the bedding planes adopted in this study were necessarily taken to be parallel to the depositional slope, the palaeosurface, and this assumption is obviously incorrect in this instance, and such an effect was not apparent in the same flow at Acquamorta (B1). In contrast the higher value of q at site A2 is associated with a clear difference between the dip of the magnetic foliation plane and the direction of dip of the palaeosurface, consistent with the predominant effect of flow stress. The same interpretation of the higher q value at site B2 is less clear as the dip of the magnetic foliation plane and of the palaeosurface are almost identical¹⁵.

This investigation, carried out on samples collected for a different purpose, has shown a surprising similarity between their fabric and that observed in sediments deposited in remarkably different conditions. Nonetheless, the consistency of these provisional observations throughout the ash layers is clear. The lowermost levels (for example, sites A1 and B1) show the effects of the palaeosurface on which they were deposited, while those from higher levels (for example, A2 and B2) show a greater importance of the current flow, implying that laminar flow may have predominated immediately before deposition, as occurs in the case of sediments. It is also interesting that the similarity of magnetic susceptibility over a distance of 3 km suggests little or no sorting of the magnetic minerals over this interval. The influence of the palaeosurface only seems to restrict the determination of the direction of flow in the basal 2 or 3 cm so that the location of their source can be attempted at higher levels. Clearly magnetic fabric studies could provide major constraints on the mechanism of deposition and mode of formation of fine grain-sized pyroclastics, although inhomogeneity problems may inhibit such studies in coarser deposits.

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The role of the geomagnetic field in the development of birds' compass sense

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The compass sense of birds is based on both celestial (Sun, sunset, skylight polarization pattern, stars) and geomagnetic cues¹⁻³. Songbirds, from birth prevented from seeing celestial cues, orient by a magnetic compass^{4,5}, and so do young homing pigeons before the development of their Sun compass^{6,7}. Furthermore, young homing pigeons exposed to aberrant solar conditions, acquire a correspondingly erroneous⁸ or incomplete⁹ Sun compass. These findings suggest that the magnetic sense may provide a primary and innate reference frame for the calibration/learning of the birds' celestial compass system^{8,9}. We have investigated the role of the geomagnetic field in the development of birds' compass sense, by shifting the geomagnetic field at pied flycatchers' (*Ficedula hypoleuca*) nests during the incubation and nestling periods. The magnetic field was shifted by Helmholtz coils mounted at the nest-boxes. A corresponding shift in the migratory orientation was observed 2 months later in the young birds' lives. This supports the magnetic calibration hypothesis, indicating imprinting-like learning at an early age of celestial compass cues used in migratory orientation.

Nest-boxes (non-magnetic material) were put up in deciduous groves close to the ecological field station Stensocka at Lund, Sweden. Their entrance holes were facing north-east (in 1981), and north-east and north-west (in 1982). Helmholtz coils (Fig. 1) were mounted on the gables of the experimental nest-boxes during the egg-laying period, and left in operation throughout the incubation (about 13 days) and nestling periods until the

evening of the 13th day after hatching (2 or 3 days before the nestlings were expected to fledge). The coils were deflecting magnetic north (mgN) 90° to the right (to geographic east) in boxes with entrance holes facing north-east, and 90° to the left (to geographic west) in boxes with entrance holes facing north-west. The vertical geomagnetic field (*Z*) was not affected, and the resulting horizontal intensity (*H*) of the right- and left-deflected fields was the same as the normal geomagnetic horizontal intensity. At Lund (1982) *H* = 17,200 nT, *Z* = 46,400 nT, *I* (angle of inclination) = 70°. The proper functioning of the coils on the nest-boxes was controlled throughout the experimental period.

Three different test categories were distinguished.

C, Controls. Young flycatchers from nest-boxes without Helmholtz coils, exposed to the normal geomagnetic field. One brood (3 young) in 1981 and three broods (4, 6, 7 young) in 1982. Because of the small number of controls in 1981, we made a few additional orientation tests with three individuals caught as resting autumn migrants.

R, Young flycatchers from nest-boxes with mgN deflected to the right, +90°. Two broods (3, 5 young) in 1981 and two broods (6, 6 young) in 1982.

L, Young flycatchers from nest-boxes with mgN deflected to the left, -90°. Two broods (6, 6 young) in 1982.

From the 13th day after hatching (taking place between 17 and 26 June for the 10 different broods), when the Helmholtz coils were dismantled, all nestlings of the different test categories were treated in an identical way. They were housed indoors in spacious cages, in the same room, with windows facing south and west, under the natural photoperiod. Orientation tests were carried out when autumn migratory restlessness was well developed, 14 August–5 September 1981 and 3 August–30 August 1982.

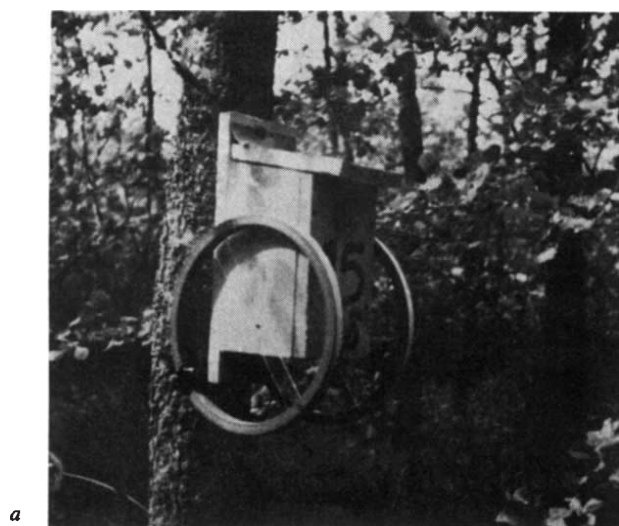
Three orientation cages (Fig. 1) were used in 1981 and four in 1982. Observers were unaware as to which test category their bird belonged, except the fourth observer in 1982, who alone selected the test birds for each night. Observations started a few minutes before sunset and lasted approximately 1 h or slightly longer. Observers recorded take-off directions of flights towards the ceiling net by birds showing migratory restlessness. Number of take-off directions (estimated to the nearest 45° compass point) registered by the observer during a test night usually were between 40 and 150 (mean close to 100), with a minimum of 13 and a maximum of 341. The mean vector for each bird in each test night was used for further analysis. On the majority of test nights, the weather was clear or only partly cloudy, admitting a good view of the skylight from the setting Sun and, during the final 30 min or so of the cage tests, of the stars.

Mean headings from all cage tests are plotted in Fig. 2. Each flycatcher was subjected to two cage tests (in 1982) or more (a

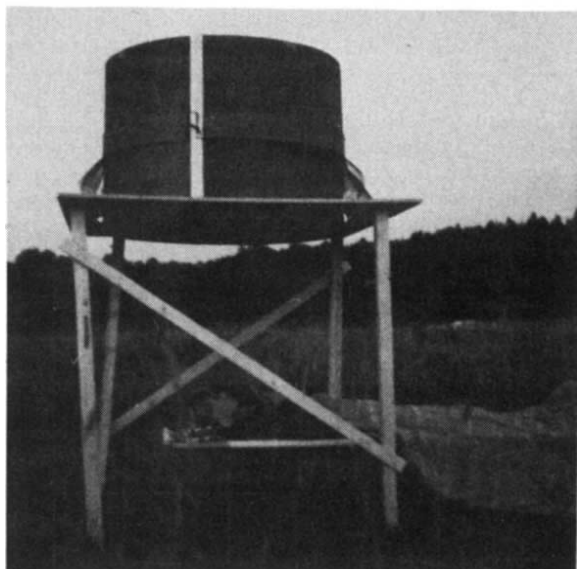
Table 1 Mean vectors calculated on the basis of mean headings from tests in orientation cages of pied flycatchers from different test categories and years

	All tests			First tests			Second tests		
	α	<i>r</i>	<i>n</i>	α	<i>r</i>	<i>n</i>	α	<i>r</i>	<i>n</i>
C (control)									
1981	317°	0.42*	(17)	289°	0.84‡	(6)	256°	0.52	(5)
1982	254°	0.17	(34)	274°	0.34	(17)	180°	0.12	(17)
1981 + 1982	289°	0.22*	(51)	281°	0.46‡	(23)	222°	0.17	(22)
R (mgN + 90°)									
1981	20°	0.56‡	(18)	8°	0.65†	(8)	59°	0.31	(5)
1982	346°	0.30	(24)	317°	0.42	(12)	28°	0.32	(12)
1981 + 1982	6°	0.40‡	(42)	343°	0.46†	(20)	37°	0.30	(17)
L (mgN - 90°)									
1982	165°	0.37†	(24)	176°	0.67‡	(12)	105°	0.16	(12)

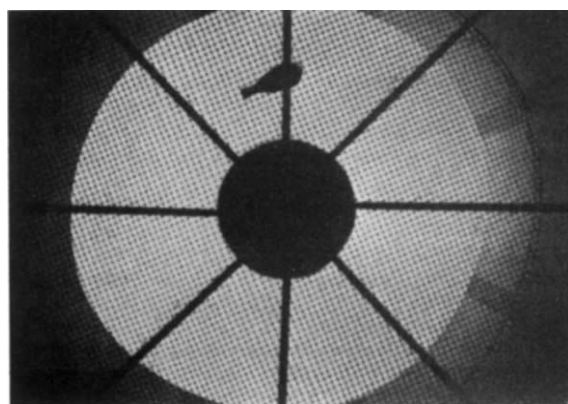
Mean vectors calculated from all tests in orientation cages are tabulated, as well as the corresponding data for the first and second test of each individual flycatcher. α = direction; *r* = vector length, *n* = number of tests. Probability of a nonrandom unidirectional distribution with *r* > 0 according to the Rayleigh test: * = 0.1 > *P* > 0.05; † = 0.05 > *P* > 0.01; ‡ = 0.01 > *P* > 0.001.



a



b



c

Fig. 1 Helmholtz coils (diameter 0.28 m; 3,100 windings per coil of diameter 0.15 mm copper wire, current from 12 V battery unit with semiconductor regulator and potentiometers) were designed by Dr Stig Borgström, Lund Institute of Technology. They were mounted on nest-boxes (a) to shift the geomagnetic field at the nests during the incubation and nestling periods of pied flycatchers. The homogeneity of the magnetic field from the coils was $< \pm 10\%$ within the nest-boxes up to at least 10 cm from the bottom. Two months later, during autumn migratory restlessness, the young birds were tested in orientation cages (b) under the natural sky at and soon after sunset. Orientation cages were circular, 1 m in diameter with a height of 0.35 m. The birds could freely fly diagonally in a cage. The floor was made out of a one-way transparent plastic film (c), permitting an observer lying underneath the cage to see the bird very clearly against the skylight, but preventing the bird from seeing the observer. The cage was covered with a rigid wide-meshed plastic net, allowing the bird (and the observer) an unhindered view of the sky overhead. A circular collar, 0.35 m high, was placed on top of the cage to screen off horizon landmarks from view, and the maximum sky sector to be seen by a bird extended 71° from zenith. Cages were oriented so that radial perches were directed along the eight compass points; the compass point of a specific perch differed in a haphazard way between the different test occasions. The start of migratory restlessness was usually easy to determine (median starting time = 11 min after sunset): the bird repeatedly 'drooped' its wings and whirled by extremely rapid wing-fluttering. Under fully developed restlessness the bird whirled continuously for long periods with the wings spread and the body held almost horizontally, turning or 'dancing' in different directions on one or more perches, before taking off in flights.

few individuals in 1981). Overall mean directions and vector lengths for the different years and test categories are summarized in Table 1. Data from first tests consistently are less scattered than from second and succeeding tests. First tests were carried out at an earlier date, most of them (46 out of 58) before 20 August, than second tests, which were practically all (51 out of 55) performed in the period 20–30 August. Flycatchers were less fat during first than second tests, and in 1982 their flight activity in the orientation cages was significantly greater in first than in second tests. A possible explanation may be that second tests coincided with a stationary and territorial period in Iberia¹⁰, 'preprogrammed' (endogenously controlled) in a circannual cycle of migratory readiness^{11,12}. The expected orientation of southern Swedish pied flycatchers is towards south-west or west-south-west, along coastal West Europe to the northwestern part of the Iberian peninsula (Swedish Ringing Scheme). Their final winter destination is tropical West Africa.

Statistical inferences are practically identical whether they are based on all available cage tests or only on first tests of each individual. On the basis of first tests, 95% confidence intervals¹³ for mean directions of the different categories are $\pm 39^\circ$, $\pm 40^\circ$ and $\pm 33^\circ$ for the C, R and L category, respectively. Comparing mean directions between the different categories by the Watson-Williams test¹³ shows that the probability that the differences are due to chance generally is very small: C versus R, $F_{41}^1 = 5.0$, $P < 0.05$. C versus L, $F_{33}^1 = 14.3$, $P < 0.001$. R versus L, $F_{30}^1 =$

30.4, $P < 0.001$. After correcting for the angular effect of the deflection of the magnetic horizontal field in the nest-boxes, indications of differences between the C, R and L samples completely vanish: C versus R -90° , $F_{41}^1 = 1.1$, not significant (NS). C versus L $+90^\circ$, $F_{33}^1 = 0.3$, NS. R versus L $+180^\circ$, $F_{30}^1 = 0.2$, NS. There was no significant difference between the orientation of one C brood from a nest-box with the entrance hole towards NW ($\alpha = 223^\circ$, $r = 0.26$, $n = 6$) and three C broods from nest-boxes with NE entrance holes ($\alpha = 277^\circ$, $r = 0.54$, $n = 14$), $F_{18}^1 = 0.9$, NS (first tests).

A process of imprinting-like intercalibration between magnetic compass cues and an unknown directional cue system, taking place in the developing eggs/nestlings in the nest-boxes, can explain these results. There are two principal possibilities of such a calibration process.

First, the unknown cue system defines the primary reference system, in relation to which a magnetic compass is calibrated, when the magnetic sense/sensor is maturing in the eggs/young birds. The magnetic compass, hence calibrated, is used as the basis for migratory orientation later in the birds' lives. According to this alternative, R birds are expected to orient at 90° to the left of controls, and L birds 90° to the right. This is clearly contradicted by the results from our experiment, and we refute this alternative of compass development.

Second, the magnetic field serves as the primary compass cue, in relation to which a secondary reference system is calibrated

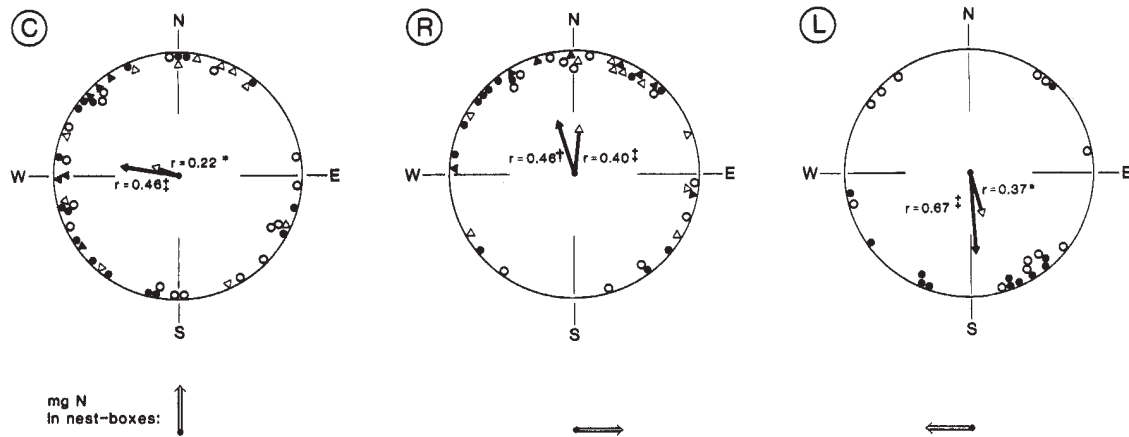


Fig. 2 Mean headings from all orientation cage tests are plotted at the periphery of the circles for three test categories of pied flycatchers: C, R and L birds from nest-boxes with the horizontal magnetic field directed as shown by the arrows below the circles. Triangles refer to data from 1981, and small circles to data from 1982. Filled symbols show results from first test of each individual flycatcher. Arrows with a filled head show mean vectors of headings from first tests; arrows with an open head show mean vectors calculated on the basis of all headings available. Probability of a nonrandom unidirectional distribution with $r > 0$ according to the Rayleigh test: * $0.1 > P > 0.05$; † $0.05 > P > 0.01$; ‡ $0.01 > P > 0.001$.

to be used for, *inter alia*, future migratory orientation. With this type of calibration process, the expected consequences for the birds' migratory orientation are in full agreement with experimental results.

The simplest assumption is that the secondary reference system is based on celestial cues. During the later phase of the nestling period, nestlings are frequently peering out from the nest-boxes' entrance holes in between the parents' feeding visits. During these observations they may imprint their compass sense, based on the magnetic field, to celestial cues. In the orientation tests, migratory restlessness started and orientation generally was established very soon after sunset, already before the stars were readily visible (at least to us) but while skylight from the setting Sun was manifest. Evidence that the magnetic compass is involved in the learning of the Sun compass has recently been reported for young homing pigeons growing up in an altered magnetic field¹⁴.

The long-lasting effect of magnetic calibration, as indicated by the present experiment, is in conflict with W. and R. Wiltschko's conclusion that migratory birds frequently recalibrate their celestial compass against the magnetic compass^{15,16}. Recalibrations may be associated with the experimental technique to call forth the use of the magnetic compass by migrants, involving long-time artificial isolation from celestial cues². The use of the magnetic compass by adult homing pigeons is called forth by special training in inclement weather¹⁷. In natural conditions, migrating birds and homing pigeons may rely on an imprinted celestial compass system (supplemented by secondarily learned celestial cues, landmarks and, possibly, wind cues¹⁸), renouncing the magnetic compass¹⁹, possibly reserving their highly sensitive magnetic sense for determination of position, rather than orientation²⁰.

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Changes in geniculate cell size following brief monocular blockade of retinal activity in kittens

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When a kitten is subjected to monocular lid suture early in life, cells in laminae of the lateral geniculate nucleus (LGN) connected to the sutured eye grow less than normal and cells in those laminae connected to the non-sutured eye grow more than normal^{1–5}. These changes are seen primarily in the binocular segment of the LGN, which corresponds to the central visual field, and are due to competition either between intracortical afferents originating from the different LGN laminae, or directly among cells within the LGN^{2,6}. The afferent deprivation induced by lid suture, however, is not complete, as retinal ganglion cells fire tonically both in darkness and in light⁷. It is generally thought that this tonic retinal activity is necessary to maintain neuronal excitability at normal threshold in the central visual pathway^{8–10}. In the visual cortex of developing kittens, we previously showed a long-lasting change in ocular dominance of binocular cells by a brief blockade of retinal activity in one optic nerve¹¹. We report here that a complete blockade of retinal activity in one eye causes major changes in LGN cell size within 1 week. These changes occur throughout the LGN, including the monocular segment where binocular competition does not occur. The results indicate that tonic retinal activity may have an important role in the control of geniculate cell size.

Nine 7-week-old kittens were used in three paradigms, three per experimental condition. The retinal blockade was induced

by two injections of 10 μ g of tetrodotoxin (TTX), a potent sodium channel blocker, into the vitreous humor of one eye, 3 days apart¹²⁻¹⁴. A single injection has been shown to silence retinal ganglion cell activity completely for roughly 3 days, during which no pupillary reflex was observed in response to light shone directly into the TTX-treated eye^{12,14}. The animals were either left in the light or placed in the dark for a week of monocular inactivation induced by TTX before being perfused with 10% formol-saline. The tissue block which contained the LGN was embedded with celloidin¹⁵, and coronal sections (40 μ m thick) were cut at regular intervals. They were then stained for Nissl substances with cresyl violet. One group of animals was studied for 3-4 weeks after the week of TTX treatment in order to determine whether the changes in geniculate cell size were reversible. The outlines of cell bodies having a visible nucleolus were first traced using a camera lucida at a total magnification of $\times 900$, and then retraced on a digitizing tablet (Tektronix 4956 graphics tablet, 4052 terminal). Seventy cells were measured from each of the most dorsal two laminae (A and A1), where corresponding regions of these adjacent laminae receive input from different eyes but from the same area of the visual field (Fig. 1a). Measurements in this binocular segment were taken from the middle of the mediolateral extent of each LGN which roughly corresponded to the central 2-5° of vision (Sanderson's map at coronal 5)¹⁶. Seventy cells were also measured from each monocular segment, the lateral extent of lamina A, which receives input from the monocular visual periphery (Fig. 1a).

The monocular inactivation induced by TTX in animals left in a lit environment produces a maximal imbalance in ascending activity from the two eyes because the untreated eye transmits both visually driven and tonic activity, while the treated eye exhibits no neuronal activity. The effects of this binocular imbalance were so strong that within 1 week the cells in laminae connected to the totally silenced eye were more lightly stained than those in the laminae which received input from the normal eye (Fig. 1b). Quantification of cell areas showed a 28% average difference (range 23-33%) in mean cell size between laminae A with visually normal input and laminae A with silenced input, a 30% average difference (23-37%) between laminae A1, and a 20% average difference (17-22%) between the monocular segments of lamina A (Table 1, Fig. 2a). The change in cell size in the binocular segment seen here after 1 week is comparable in magnitude with the change seen in kittens subjected to monocular lid suture from birth for several months^{3,5}. Additionally, although changes in cell size in the monocular segment of lid-sutured kittens have been reported previously, they were of smaller magnitude and required much more time to be expressed^{3,5}. The difference in cell size between the monocular segments of the TTX-treated kittens was substantial, but consistently smaller than between the binocular segments. Since no binocular competition is likely to occur in the monocular segment, the change in cell size seen in this segment should be due exclusively to the silencing of retinal activity. The magnitude of the difference in cell size between monocular segments was roughly two-thirds of that obtained from the binocular segments (20% versus 28-30%). This suggests that much of the change obtained in the binocular segments of these TTX-treated animals was due to the absence of retinal activity, rather than binocular competition, the major factor in monocularly lid-sutured animals.

By placing animals in the dark during the week of monocular TTX treatment, we further assessed the relative importance of tonic retinal activity alone on changes in cell size. The binocular imbalance produced in these animals was due to the presence of only tonic afferent activity in one optic nerve and no such activity in the other optic nerve, there being no visually evoked activity in the dark. This somewhat subtle binocular imbalance still resulted in significant changes in cell size (Table 1, Fig. 2b). The average difference in mean cell size between the laminae A which received only tonic retinal activity and those with no activity through the optic nerve was 17% (range 13-20%);

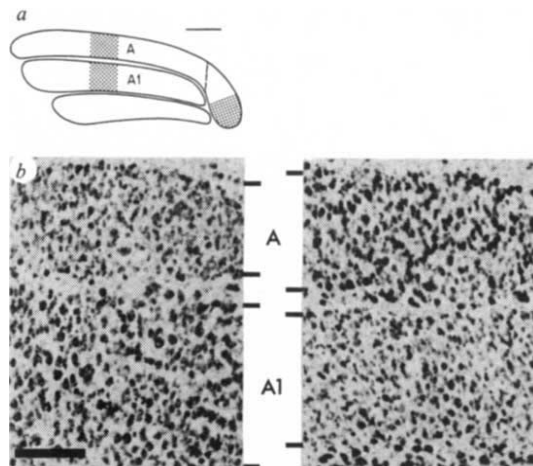


Fig. 1 a, A schematic drawing of a coronal section through the middle of the dorsal lateral geniculate nucleus (LGN) of a kitten. Medial is to the left, dorsal is up. Lamina A receives input exclusively from the contralateral eye and lamina A1 from the ipsilateral eye. Where they lie adjacent, corresponding regions of each lamina receive input from the same area of visual space. The lateral extent of lamina A is usually referred to as the monocular segment (MS), since it receives input only from the monocular visual periphery. The stippled zones indicate the sites of cell size measurement. The dotted line represents the border between the monocular and binocular segments of lamina A. Scale bar, 500 μ m. b, Photomicrographs of coronal sections of laminae A and A1 from each hemisphere of a 7-week-old kitten subjected to 1 week of monocular retinal blockade induced by intravitreal injections of tetrodotoxin (TTX). Cells in laminae connected to the silenced eye (lamina A at left, lamina A1 at right) are noticeably smaller and more lightly stained than in their corresponding laminae connected to the normal eye (lamina A at right, lamina A1 at left). Scale bar, 200 μ m.

between laminae A1 it was 17% (14-21%) and between the monocular segments, 18% (17-19%). The silencing of tonic retinal activity alone, therefore, produced a significant and relatively uniform change of cell size throughout the LGN. The change in size in the binocular segment of the LGN was not greater than in the monocular segment, in contrast to what was observed in the TTX-treated animals left in the light. The magnitude (17-18%) of the changes in this condition was close to that seen in the monocular segment of animals kept in the light (20%). These observations imply a common cause for the changes in cell size throughout the LGN in the dark and those in the monocular segment in the light and suggest that both changes were due to the loss of tonic retinal input but not to any form of competitive interaction or the lack of visual activity itself. Furthermore, as the kitten was 7 weeks old when the TTX treatment was started, it is most likely that the quick change in geniculate cell size revealed here was not due to the cessation of normal growth⁵. Rather, it may be a case of cell shrinkage due to the lack of tonic afferent activity. At any rate, we are currently investigating whether these changes in cell size are due to hypertrophy in the active laminae with normal visual input or atrophy in the inactive laminae with totally silenced input.

The TTX treatment appears to be nondegenerative, in that the rapidly induced changes in geniculate cell size are reversible. Within 3-4 weeks after the end of the week of TTX treatment a much smaller difference in cell size was observed between laminae with previously silent input and laminae with normal input (Table 1, Fig. 2c), although lamina A1 appeared to recover more rapidly than other regions in the LGN.

Thus, by totally blocking tonic retinal activity in one eye of 7-week-old kittens for 1 week, significant changes in geniculate cell size were seen throughout the LGN. These changes were obtained in conditions otherwise not conducive to rapid cell size change: in the monocular segment of animals left in the light,

Table 1 Mean $\pm$ s.d. (μm^2) of laminar cell size of all experimental animals

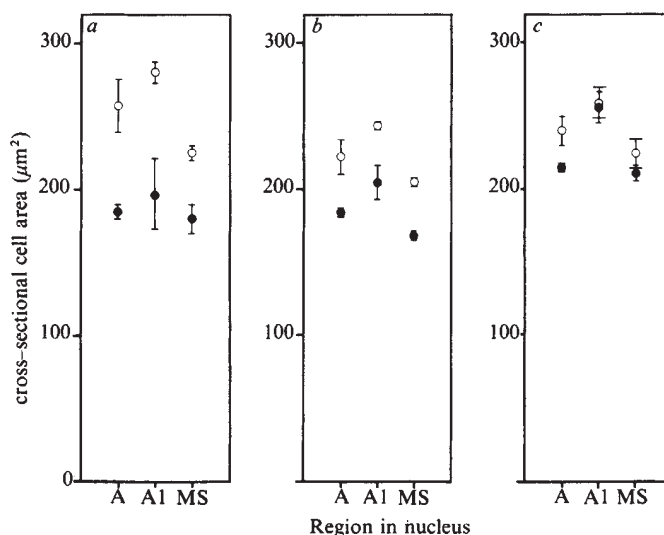
Animal	Treatment	A			A1			MS		
		Inactive	Active	%Δ	Inactive	Active	%Δ	Inactive	Active	%Δ
TT1	TTX-light	186 ± 42	278 ± 67	33%	201 ± 50	284 ± 75	29%	189 ± 42	228 ± 59	17%
TT3		180 ± 42	249 ± 67	27%	171 ± 42	272 ± 59	37%	170 ± 42	218 ± 50	22%
492		190 ± 42	246 ± 67	23%	219 ± 59	285 ± 59	23%	181 ± 33	226 ± 42	20%
Grand mean ± s.d.		185 ± 4	258 ± 14	28%	197 ± 20	280 ± 6	30%	180 ± 8	224 ± 4	20%
327	TTX-dark	181 ± 33	215 ± 42	16%	206 ± 33	244 ± 59	16%	167 ± 42	206 ± 42	19%
331		188 ± 42	236 ± 50	20%	192 ± 42	243 ± 59	21%	169 ± 42	203 ± 42	17%
481		187 ± 42	215 ± 50	13%	215 ± 50	250 ± 59	14%	168 ± 33	202 ± 42	17%
Grand mean ± s.d.		185 ± 3	222 ± 10	16%	204 ± 9	246 ± 3	17%	168 ± 1	204 ± 2	18%
TT5	Recovery	216 ± 50	245 ± 84	12%	246 ± 67	249 ± 67	1%†	216 ± 50	232 ± 50	7%*
560		216 ± 59	248 ± 59	13%	265 ± 67	270 ± 75	2%†	209 ± 42	226 ± 59	8%*
592		210 ± 50	231 ± 50	9%*	257 ± 67	254 ± 67	1%†	204 ± 50	212 ± 59	4%†
Grand mean ± s.d.		214 ± 3	241 ± 7	11%	256 ± 8	258 ± 9	1%	210 ± 5	223 ± 8	6%

Each value is the mean $\pm$ s.d. of 70 cell areas from the indicated lamina of a single animal, given in μm^2 . Inactive refers to laminae with input from the TTX-treated eye; active refers to untreated laminae. The % Δ was determined by dividing the difference in mean cell area between corresponding active and inactive laminae of a single animal by the mean cell area of the active lamina. Absence of asterisks above the % Δ indicates a statistically significant difference at $P < 0.001$. Treatment code is as follows: TTX-light refers to 1 week of monocular TTX-induced inactivation beginning at 7 weeks of age while the animal was left in a normally lit environment. TTX-dark animals were treated similarly with the exception that during the week of TTX treatment the animal was placed in complete darkness. Recovery animals were first subjected to 1 week of monocular TTX treatment beginning at 7 weeks of age and then allowed 3 weeks of recovery from the TTX treatment.

* Differences significant at $P < 0.05$.

† Differences that were not significant at $P < 0.1$.

Fig. 2 The grand means of geniculate cell areas in 7-week-old kittens subsequently subjected to 1 week of monocular TTX treatment. In the normal LGN, cells in lamina A1 are slightly larger than cells in both the binocular and the monocular segments of lamina A, so the appropriate comparison of cell size is between corresponding segments and laminae from the two hemispheres. Laminae with normal input are indicated by open circles and those with input from the silenced retina are indicated by filled circles. Each point represents the grand average of the three means of cell size obtained from three animals in the same experimental condition. Vertical bars represent double standard deviations. Statistical comparisons between kittens were made by treating the mean cell size of each lamina as a single observation and then using a paired t -test on the means from corresponding laminae of the three animals in each experimental condition. The percentage difference was determined by dividing the difference in mean cell size of corresponding laminae by the mean cell size of the lamina with input from the normal eye (see Table 1). **a**, Changes in geniculate cell size after 1 week of monocular TTX treatment in a lit environment. We observed a 28% average difference in cell size between laminae A with normal input and those connected to the silenced eye ($t_2 = 6.8$, $P < 0.025$), a 30% average difference between laminae A1 ($t_2 = 7.99$, $P < 0.01$), and a 20% average difference between the monocular segments ($t_2 = 16.63$, $P < 0.005$). The change in cell size was significantly larger in the binocular segment, where both tonic retinal activity and binocular competition can affect cell size, than in the monocular segment, where a basis for binocular competition does not exist (t -test for independent measurements using the percentage difference per region as a single observation; d.f. = 7, $t = 2.16$, $P < 0.025$). **b**, Changes in geniculate cell size after 1 week of monocular TTX treatment while the animals were kept in the dark. The difference in cell size between corresponding regions was 17% in lamina A ($t_2 = 6.19$, $P < 0.025$), 18% in lamina A1 ($t_2 = 8.42$, $P < 0.01$) and 18% in the monocular segment ($t_2 = 21.4$, $P < 0.005$). Note that the change in cell size is relatively uniform throughout the LGN, as compared with what was seen in **a**, suggesting that binocular competition did not have a role in the changes observed here. **c**, Within 3–4 weeks in the light after the end of the week of monocular TTX treatment (also while in the light), the difference in cell size between corresponding laminae was greatly reduced. Accordingly, the mean cell size showed an 11% difference between laminae A, a 1% difference between laminae A1 and a 6% difference between the monocular segments. While the recovery of cell size in the binocular and monocular segments of lamina A was not complete, a comparison of the percentage difference in corresponding regions between control (TTX-treatment only) and recovery animals indicated that the recovery was significant in these regions (t -test for independent measures using the percentage difference per region as a single observation, d.f. = 4; $t_A = 5.19$, $t_{MS} = 7.07$; $P < 0.005$ in both regions).



and throughout the LGN of animals placed in the dark during the week of retinal blockade. The present results indicate that changes in geniculate cell size are controlled by at least three factors: tonic retinal activity, which can elicit strong changes throughout the LGN and appears to be the most significant factor in this study; binocular competition, which affects only cells in the binocular segment², and visually evoked afferent activity *per se*, which produces only minor changes in cell size⁶. The relative contribution of these three factors, however, might

be different from that described here if observed for a much longer period than 1 week or at a different stage of development. The present findings are related to recent reports which have demonstrated that blocking tonic retinal input results in the abnormal development of geniculate physiology¹⁴ as well as the lack of anatomical segregation of the ocular dominance columns in the visual cortex¹³. Taken together with previous findings^{9–14}, the present results are consistent with the hypothesis that tonic retinal activity has a significant role in both the development

and the subsequent maintenance of normal connectivity in the mammalian central visual pathway.

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Electrophysiological correlates of hyperacuity in the human visual cortex

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The human visual system is capable of detecting a vernier misalignment with extraordinary accuracy. Since this remarkable precision in spatial localization is better than can be naively predicted by simple optical or anatomical considerations it has been termed a hyperacuity¹. So far no single neurone model seems capable of accounting for hyperacuity², and the retinal image might require reconstitution in a finer grained form in the visual cortex³. We report here an electrophysiological correlate of hyperacuity recorded from the human visual cortex. The amplitude of the visually evoked potentials (v.e.ps) elicited by the appearance of a vernier offset varied systematically with the magnitude of the offset. Extrapolation of the function relating v.e.p. amplitude and log offset to zero voltage resulted in an electrophysiological estimate of vernier acuity that was similar to the observer's psychophysical threshold.

The stimuli in these experiments were repetitive vernier patterns generated on the monitor of a small computer, and are shown schematically in Fig. 1 (top). Specifically, the stimulus consisted of five bright horizontal lines, each composed of three segments, presented on a dark background. Initially all of the line segments were aligned. The appearance of the vernier offset was introduced by displacing the outer line segments downward; after 350 ms the outer line segments were returned to their original aligned position (disappearance). In this manner, the appearance of the stimulus changed from colinearity of the line segments, to non-colinearity without changing the mean luminance of the display.

The observer's electroencephalogram (EEG) was amplified (10^5 times with a pass band of 0.3 to 100 Hz), and time-lock averaged in synchrony with the appearance of the vernier offset. Figure 1 shows the responses elicited by offsets of different magnitudes for two observers. The v.e.p. elicited by the vernier offset was characterized by a positive going wave with an onset roughly 100-110 ms after the appearance of the vernier offset. The response was quite reliable (Fig. 1), and the amplitude of

the positive deflection varied systematically with the magnitude of the offset. It is well known that accelerated motion (displacement) of a pattern across the retina can evoke scalp potentials⁴. Therefore, in order to demonstrate a visually evoked potential correlate of vernier acuity, it is important to ensure that the responses obtained were not evoked by displacement (motion) of the retinal image. To control for this displacement artefact, we recorded responses elicited by an equal displacement of all the line segments moving together. Psychophysically, the human observers' sensitivity to unreferenced motion is 7 to 10 times poorer than their sensitivity to a vernier offset⁵. Thus, as expected, in the present study the displacement of all of the line segments by 40 s was subthreshold, and resulted in an evoked potential which could not be distinguished from noise (Fig. 1, bottom right). In contrast, a vernier offset of 40 s resulted in a robust and reliable response. Therefore, we conclude that in the range of vernier offsets near threshold (10 to 50 s) displacement artefacts do not contribute to the responses. It is also of interest that the waveforms elicited by the vernier display differ from those elicited by other patterned stimuli. For comparison, responses elicited by the appearance/disappearance of a 15 cycle per degree square wave grating recorded under the same stimulus and recording conditions are shown for observer K.B.S. (Fig. 1, bottom left). The responses elicited by the appearance of the pattern differ from the vernier v.e.p. in showing an early negative deflection and shorter peak latencies. It is interesting that the waveforms elicited by the appearance of a vernier offset

Table 1 V.e.p. vernier thresholds obtained with two different electrodes

Observer	V.e.p. threshold (arc s)		Psychophysical threshold† (arc s)
	Right to midline electrode	Left to midline electrode	
K.B.S.	8.1	7.2	7.5
B.W.Z.	15.4	10.5	16.6
D.M.L.*	8.15	7.15	6.3
R.E.M.*	9.3	10.75	13.6

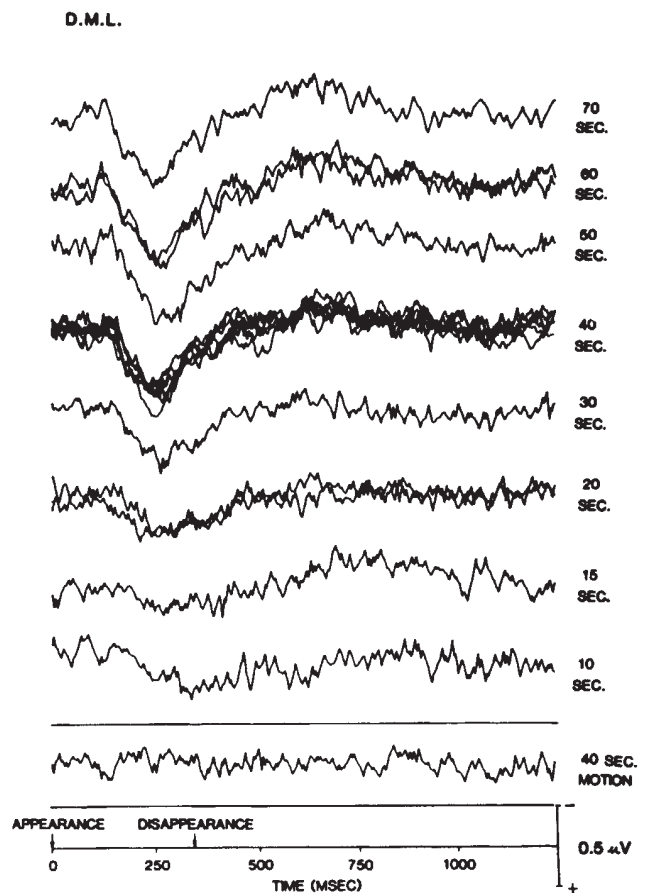
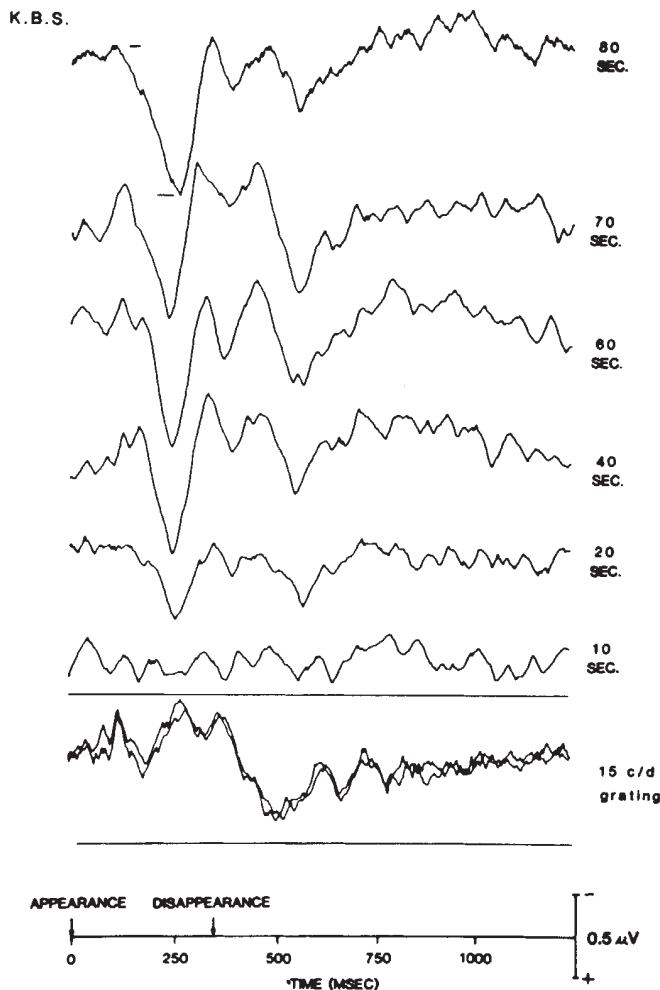
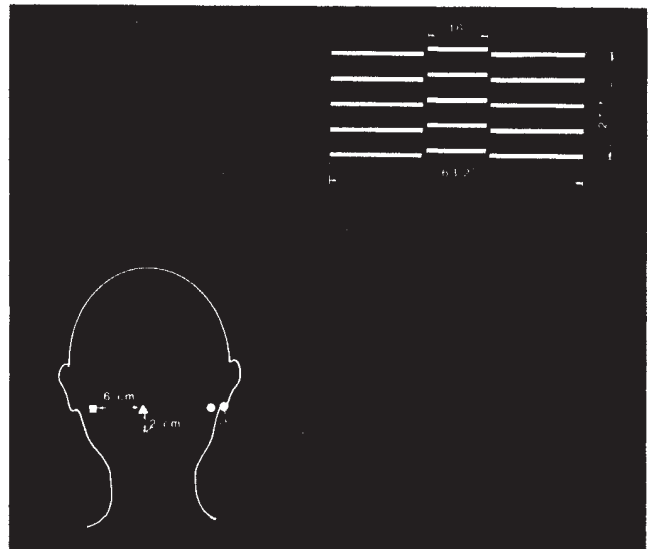
*Mean of two threshold estimates.

† 75% seen (yes/no method of constant stimuli). The false alarm rate was 4 to 9%.

are reminiscent of the waveform elicited by another type of hyperacuity stimulus, stereopsis. Regan and Spekreijse recorded visually evoked potentials elicited by the appearance of a change in depth⁶. The waveforms of their v.e.ps were elicited by disparities much larger than the offsets used here, but are at least qualitatively similar to those shown in Fig. 1.

The v.e.p. amplitude varies systematically with the magnitude of the vernier offset. When the amplitude is plotted as a function of log offset (Fig. 2), the results are well fit (correlations were always greater than 0.93) by a line of the form $Y = M \log (X/X_0)$ for X greater than X_0 and $Y = 0$ for X less than X_0 . Campbell and Maffei⁷ have used the method of extrapolation to zero voltage to obtain an electrophysiological contrast 'threshold' estimate. Our electrophysiological estimate of vernier threshold, X_0 (shown by the intercept of the line with the abscissa) has a standard error of about 20% (shown by the horizontal error bar), and is in reasonable agreement with the psychophysical estimate of threshold (shown by the arrow for each observer). Table 1 summarizes the 'v.e.p. vernier thresholds' obtained with two different electrode configurations (shown in Fig. 1). Psychophysical thresholds were also obtained for each of the four observers. A yes-no method of constant stimuli with feedback in which the stimulus was presented with one of four offsets (including blanks) was used to obtain psychometric functions for vernier detection. Interpolation to the 75% seen level provided a psychophysical estimate of threshold, which is also shown in Table 1.

Fig. 1 Top: Schematic illustration of the stimulus and recording conditions. The stimulus, which subtended about 1° by 0.5° , consisted of 5 rows of bright lines each of 3 segments. Before introduction of vernier offset all segments were aligned. Vernier offset was introduced with no change in luminance, by displacing the lateral segments downward (appearance of vernier offset) and back (disappearance of vernier offset) at a rate of 0.71 Hz. The centre segments aided accommodation and fixation. V.e.ps were recorded with a bipolar electrode arrangement. At least two different electrode pairs were used. All the data shown here are for the electrode pair illustrated by the $\circ$ and Δ (2 cm above theinion on the midline, and 6 cm to the right). Table 1 also includes results obtained with a second electrode pair ($\square$ and Δ). For both electrode pairs the midline electrode served as the reference. Bottom: Averaged v.e.ps ($n = 300$) elicited by the appearance/disappearance of vernier offsets ranging from 80 to 10 s of arc are shown for observers K.B.S. (left) and D.M.L. (right). Several examples of repeated runs are shown to demonstrate the reliability of the data. The six traces shown for D.M.L. (40 s offset) were recorded on two different days. The two responses shown for D.M.L. to 60 and 20 s offsets were recorded during the same session. For comparison, v.e.ps recorded in identical conditions in response to the appearance/disappearance of a 15 cycle per degree (120 s per bar) squarewave grating are shown for observer K.B.S. Also shown are the responses recorded to a displacement of all of the line segments (motion) of 40 arcs, for D.M.L. In contrast to the highly visible vernier offset of 40 s, the unreferenced motion was subthreshold and resulted in responses which could not be distinguished from noise.



Vernier thresholds depend critically upon the relative distances between the features of the stimulus¹. This property of vernier acuity is also evident in the v.e.p. Figure 3 shows how the v.e.p. amplitude varies as a function of offset magnitude for three different conditions, with the features abutting, separated by 7.5 min or with no reference lines (displacement). The psychophysical thresholds obtained in identical conditions are shown by the arrows, and are in good agreement with the electrophysiological estimates. This dependence of the v.e.p.

amplitude on the proximity of the stationary reference is similar to that recently reported by Zemon and Ratliff⁸ which they interpreted as a demonstration of lateral interactions.

We conclude that the responses obtained in these experiments were elicited by the appearance of the relative positional information in the vernier stimulus introduced by breaking colinearity. To our knowledge, this is the first report of evoked potentials elicited by such localized changes in the appearance of a stimulus. This is of particular interest since the v.e.p.

Fig. 2 The peak-to-trough amplitude of the early components elicited by the appearance of the offset (shown by the horizontal lines on the top record of K.B.S. in Fig. 1) are plotted as a function of the magnitude of the offset. Note that offset is plotted on a logarithmic scale for each of the four observers. The lines were fit to the data by nonlinear regression. The v.e.p. vernier threshold was given by the parameter X_0 of the nonlinear regression. The horizontal error bars show ± 1 s.e. of the v.e.p. threshold. For comparison, psychophysical thresholds (75% detection level) are indicated by the arrows. For observers D.M.L. and R.E.M., two sets of data are shown. The results shown by the filled symbols and solid lines are with a repetition rate of 0.71 Hz. The data shown by the open symbols and dashed lines were obtained with a higher repetition rate (1.42 Hz) for R.E.M. For D.M.L., the open symbols and dashed lines were obtained in response to the appearance of a vernier offset for 350 ms every 1,400 ms (0.71 Hz) followed 700 ms later by a displacement of all of the line segments (motion) of the same magnitude.

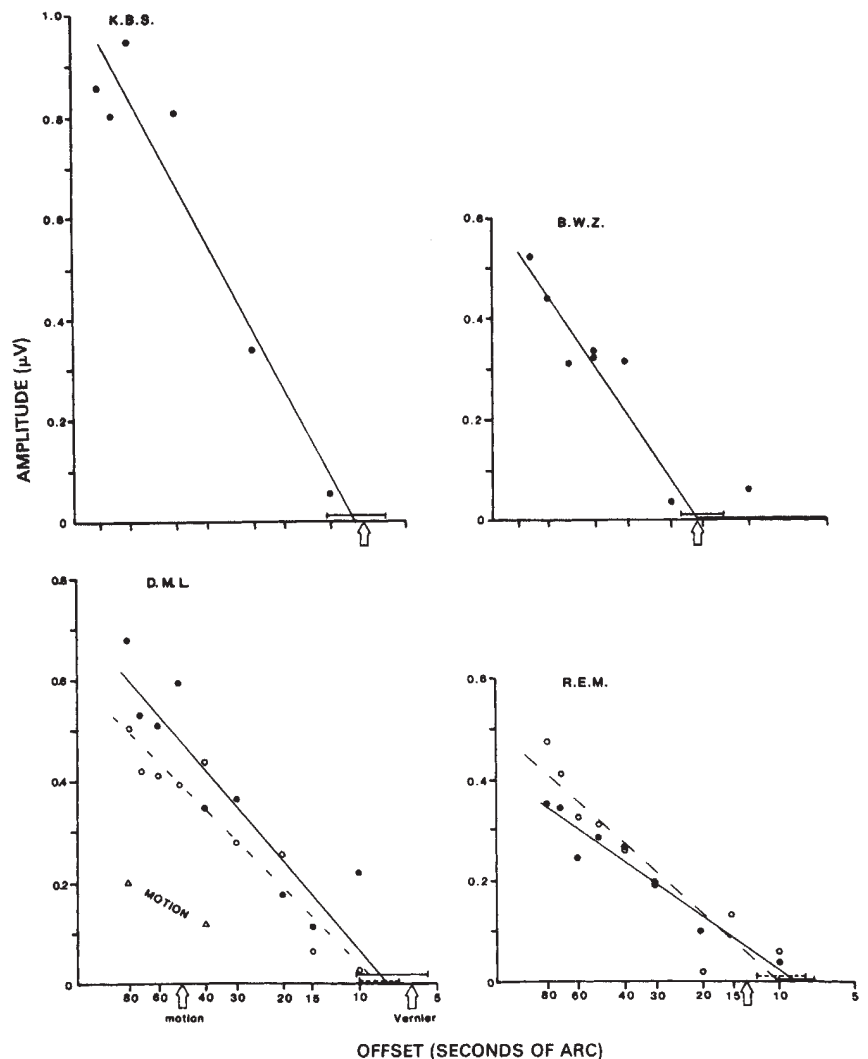
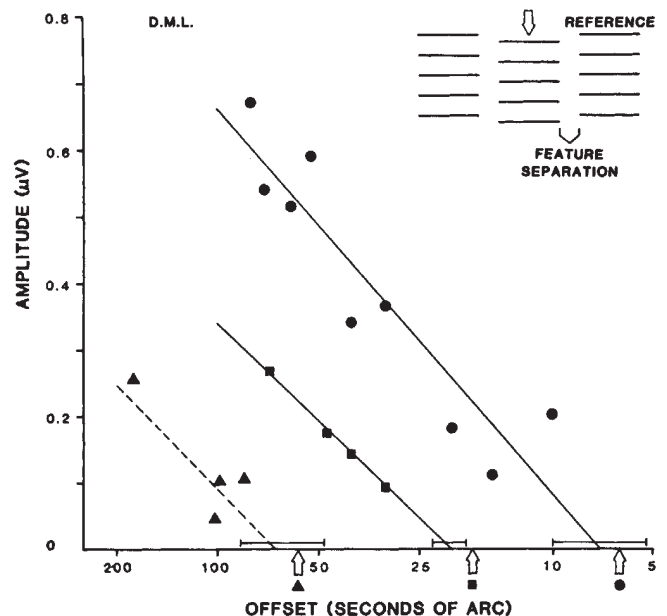


Fig. 3 Fine spatial localization depends strongly on the proximity of the reference features. In order to determine whether this property of vernier acuity is reflected in the v.e.p., responses elicited by the displacement of the *central* line segments were recorded, while the lateral (reference) line segments were separated at various distances from the central test lines. V.e.p. amplitudes as a function of the magnitude of the offset for no feature separation ($\bullet$), 7.5 minute separation ($\blacksquare$), and with no reference lines ($\blacktriangle$) are shown for D.M.L. The lines are fit to the data by regression. The open arrows show the psychophysical thresholds obtained under the same conditions.



provides a new tool for investigating hyperacuity. In addition, it seems likely that these, and other hyperacuity stimuli, may provide a very sensitive technique for the investigation of anomalies of spatial vision⁹.

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Cell lineage and the induction of second nervous systems in amphibian development

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The amphibian nervous system has long been thought to arise from within a large ($>10^3$) population of dorsal ectodermal cells¹, that would otherwise differentiate only as epidermis, as a result of inductive signals from underlying dorsal mesoderm at gastrulation². It has recently been claimed, however, that small cell groups are set aside much earlier in amphibian development, as the sole founders of particular portions or 'compartments'³ of the body plan⁴⁻⁶. This would imply a dramatic re-interpretation⁵ of classical experiments where a second dorsal blastoporal lip, grafted to the ventral side of a gastrula containing 10,000 or more cells, causes there the development of a second central nervous system (CNS) in host tissue⁷⁻⁹. We show that such surgically induced second nervous systems are made by host cells which have lineages separate from those that contribute to the original CNS from the 32-cell stage. Thus neural induction can occur as traditionally supposed, by assignment of ectodermal cell fate in relation to dorsal mesoderm during gastrulation. We discuss the implications of this for the recent proposals about early compartmentation in vertebrate development.

As a lineage-linked cell marker, we used dextran (MW ~10,200) covalently attached to fluorescein (1:1 molar ratio), for visualization by fluorescence microscopy, and to lysine (approximate 3:1 molar ratio), to ensure co-fixation with protein in buffered formaldehyde fixative (R. Gimlich and J. Braun, in preparation). For details of blastomere injections and subsequent processing of embryos, see the legend to Fig. 1.

In fertilized *Xenopus laevis* eggs, the sperm entry point is visible before cleavage. In many embryos, the meridian through this point corresponds both to the future ventral midline and to the first cleavage plane. Particular blastomeres in the four tiers of eight which comprise the 32-cell morula, in this cleavage pattern, populate reproducible domains within the later body pattern (stage 28–30¹⁰; 2½ days development at 20 °C). We have chosen such embryos for our work. Other cleavage configurations frequently accompany normal development.

In separate samples of embryos at the 32-cell stage, we filled individual blastomeres near the prospective dorsal or ventral midlines. Blastomere D2 is in the second tier on the dorsal side, and blastomere V3 in the third tier on the ventral side (Fig. 1A). The progeny of these cells populate domains just above the dorsal or ventral marginal zones at gastrulation. Such embryos were allowed to develop to the early gastrula (stage 10), when a second dorsal blastoporal lip, from a synchronous but entirely unlabelled donor, was implanted mid-ventrally under the marginal zone. Unoperated embryos, filled at either blastomere position, served as controls for mapping the domain normally populated by the respective descendant cells in larvae. The operation, the approximate distributions of marked cells in early gastrulae, and a schematic view of the alteration to the normal course of development after organizer implantation, are shown in Fig. 1B–D.

Second nervous systems arising in the presumptive belly region of operated embryos are typically associated with a

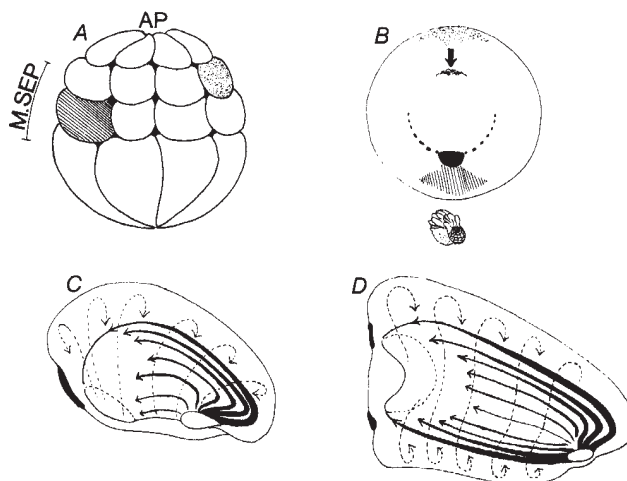
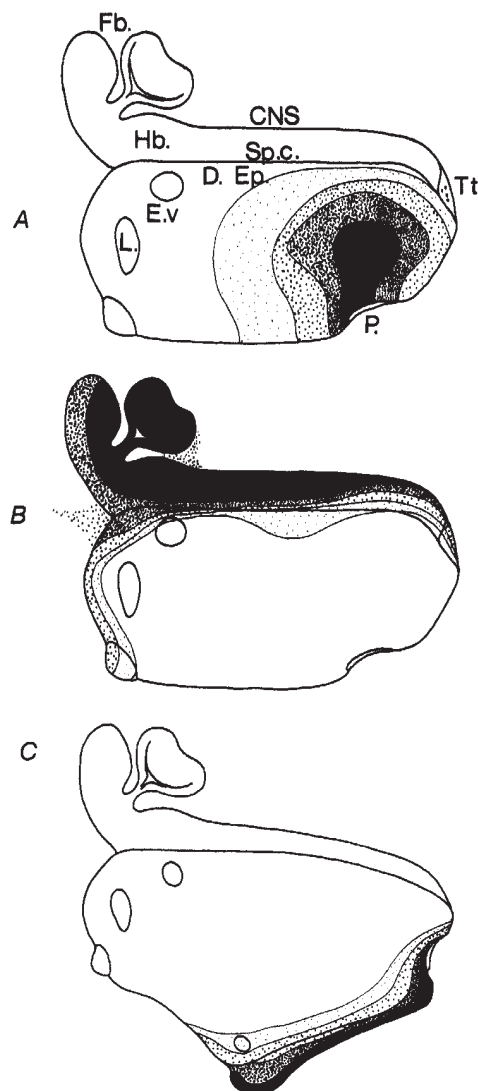


Fig. 1 Blastomere marking and the implantation of second organizers. **A**, View from the left side of the typical 32-cell blastomere array, approximately bilaterally symmetrical about a plane containing the animal and vegetal poles and the sperm entry point (SEP). The position V3 is shown cross-hatched, and position D2 stippled. Eggs were held in a given orientation with respect to the SEP and, at the 32-cell stage, blastomeres were injected with about 0.5 nl of fluorescein-lysine-dextran at 100 mg ml⁻¹ in water. Marker was delivered using an air-pressure system in which flow rate from the micropipette tip (2–5 µm diameter) was calibrated before each few injections. Embryos were allowed to develop to stage 28–30¹⁰, then fixed in 4% formaldehyde in 0.1 M cacodylate buffer, pH 7.4. Fixed embryos were rinsed, dehydrated through 100% ethanol, and embedded in JB-4 (Polysciences Inc.) for sectioning at 5 µm. M, SEP, meridian of sperm entrance point; AP, animal pole. **B**, View from vegetal (future yolk plug) aspect of the beginning gastrula stage. The dorsal midline and position of the normal dorsal blastoporal lip are marked with an arrowhead. The pocket excavated in the line of the future mid-ventral blastoporal lip into which a dorsal fragment is grafted, is shown in solid black^{8,14}. The present operations were performed in 66% modified Ringer solution at pH 7.2. The ionic strength was lowered to 25% after 40 min healing, and subsequent development was at 20 °C. The size of the graft used is shown approximately to scale, below the host. Hatched and stippled areas show the approximate surface domains occupied at this stage by labelled descendants of V3 and D2, respectively, though the D2 domain also extends further towards the animal pole (out of view in the diagram). **C**, **D**, Cumulative movements of mesodermal and neurectodermal layers by the period of late gastrulation and neural tube formation: **C**, normal development; **D**, after successful mid-ventral organizer grafting (J.C., unpublished observations). The graft supplies a second dorsal midline for patterning, antero-posterior extension and dorsal convergence movements in the cylindrical mesodermal mantle, while a second area of columnarization and inrolling to form a neural tube occurs in the ectoderm. Twinning occurs without any enhanced mitosis¹⁴. Mesodermal extent and movements are represented by solid black lines, with thickness proportional to degree of convergence, antero-posterior extension, and 'dorsal' differentiation. Material for the posterior dorsal mesoderm normally sweeps dorsally into the tailbud region from a recruitment zone around the blastopore, to take its place in the axis. Dotted lines represent convergence and inrolling by the ectoderm in the formation of neural structures. The morphogenesis is viewed from the posterolateral aspect, with the ventral side rolled slightly towards the observer. For details of the fate and movements of cell layers see refs 15 and 16.

second edition of the mesodermal pattern, in a mirror-image relationship to that around the host dorsal midline (Fig. 1C, D). We discuss here only the clonal origins of ectodermal cells contributing to the normal nervous system and to that induced by the grafted organizer in experimental embryos. The size of labelled mesoderm populations appeared little affected by our twinning operation (data not shown), so that organizer implantation into gastrulae does not cause many cells to change their germ layer membership.

Fig. 2 Distribution at tailbud stage for the neurectodermal descendants of 32-cell blastomeres in the left half-embryo's total ectodermal surface at stage 28–30: **A**, the V3 blastomere in normal development, compiled from 12 embryos; **B**, the D2 blastomere in normal development, compiled from seven embryos; **C**, experimentally altered development with median observed grade of second nervous system, as populated by V3 descendants. CNS patterns are drawn in continuity with the epidermis, and unfolded along the mid-dorsal line of earlier neural tube closure. Though most filled blastomeres preferentially populate one side or the other of the larval neurectoderm, we observed considerable mixing of V3 descendants across the ventral midline, and of D2 descendants across the forebrain floor and eyes^{4–6}. These maps depict the summation of single fills populating left and right sides of larvae, and thus define zones of % probability of encountering V3 or D2 descendants. The degree of cell mingling (that is, the % single-labelled cells) in any region was generally inversely proportional to the probability of incidence of labelled cells.

Methods: Transverse sections at forebrain and eye, hindbrain and ear vesicle levels, and at regular intervals back to tailtip, ten to twelve sectional levels in all, were examined at X160 under epifluorescence optics (excitation at 450–490 nm, barrier filter at 520 nm). Profiles were drawn and positions and sizes of labelled ectodermal cell groups marked. The epidermis at each level was divided into eight equal sectors from dorsal to ventral, and the wall of the CNS into four such sectors. The % scores for presence of descendants in each sector at each level throughout the series of embryos were plotted to give the distributions shown. **A**, anterior; **D**, dorsal; **Ep.**, boundary of epidermis; **CNS**, boundary of central nervous system; **L**, epidermal lens placode; **E.v.**, epidermally derived ear vesicle; **Fb.**, forebrain and eyecup; **Hb.**, hindbrain; **Sp. c.**, ectodermally derived spinal cord; **Tt.**, tailtip spinal cord; **P**, proctodaeum or original blastopore. Anterodorsal external stippling, head neural crest. Solid black zone represents $\geq 80\%$ incidence, and successively lighter zones of stippling represent 50–80%, 20–50%, and $\leq 20\%$ incidence of labelled cells.



Twelve control and 10 experimental embryos were analysed after filling of blastomere V3, while seven control and seven experimental examples were examined after fills of blastomere D2. Animals were from three egg batches, each contributing controls and experimentals. Most of the induced neural systems, although quite large, lacked structures anterior to the ear vesicle and hindbrain, but in two each of the V3 and D2-filled experimentals all the principal CNS regions were present overlying appropriate mesodermal structures.

The filled ventral and dorsal blastomeres in normal development populated, respectively, an ectodermal domain that never participated in nervous system development and one that always made a major contribution to it. Figure 2A and B shows spatial maps, constructed from transverse sections of control larvae, of the frequency with which V3 and D2 descendants, respectively, are found in the ectodermal derivatives of the normal larva. The total neurectodermal territory at the tailbud stage is treated as a topologically continuous sheet, as is the case until segregation of the rolled-up neural tube from the epidermis at the neurula stage. For each blastomere fill, we observe, as would be expected, labelling of almost every cell in a central region of ectoderm (Fig. 1B) surrounded by a much larger, but still restricted region containing scattered labelled cell groups and single labelled cells: this is presumably the result of progressive, diffusion-like mixing of cells, beginning at an early stage^{5,6}.

When the V3 blastomere was filled, the labelled domain in control larvae was centred on the rear belly and ventral tailbud, extending at reduced density anterior and posterior to the blas-

topore, and up the flanks (Fig. 2A). The domain never extended far enough dorsally to have contributed to the neural plate. In only two out of twelve control embryos, were a few isolated labelled cells observed in tailtip spinal cord, which is believed to have a distinct origin in terms of the traditional germ layers¹¹. By contrast, in every V3-filled experimental embryo, the second CNS was populated with labelled cells at high or moderate frequency through much of its extent (Fig. 2C). The size of the tailbud was characteristically reduced compared to controls, and the domain of labelled epidermis longer, narrower and less dense. Profiles typical of all major brain levels were seen populated with V3 descendant cells in experimental animals (Fig. 3A, B), and at spinal cord levels where neurogenesis is precocious, differentiation of typical neurogenic epithelium and neurites was visible (Fig. 3C, D). This clearly shows that ventral presumptive epidermal cells differentiate as elements of the nervous system if underlain by dorsal mesoderm.

Filling of blastomere D2 labelled a neurectodermal domain centred in forebrain and eye regions of the normal CNS (Fig. 2B). The more posterior neural axis was also labelled, especially in its ventral regions. D2 descendants sometimes populated head epidermis and, as would then be expected, head neural crest. There was often a labelled epidermal domain along the extreme dorsal midline, but sometimes the entire marked population was removed from the body surface by neural tube closure. No second nervous system appeared to contain any descendants of the filled D2 blastomere, although original 'host-organized' nervous systems were all filled as in controls. Thus, cells fated

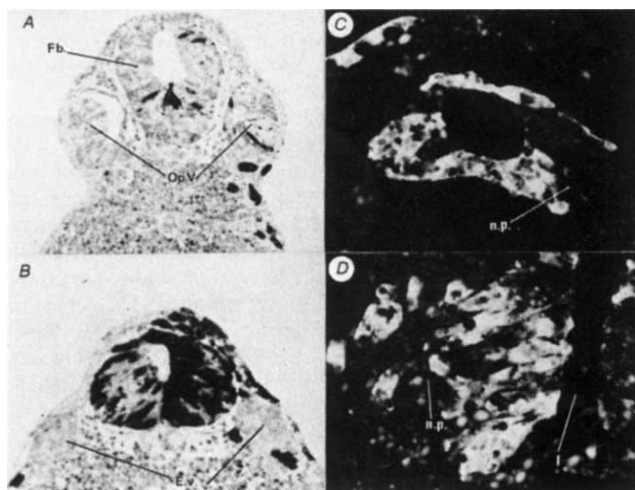


Fig. 3 Labelled cells in second nervous systems of experimental embryos filled at position V3 at the 32-cell stage. **A, B**, Negative photographic images of 5 µm transverse sections of two experimental V3-filled embryos at stage 28-30. Labelled V3 descendants (black profiles) populate the forebrain and optic vesicle (**A**), and the hindbrain at the ear vesicle level (**B**) of the second axes. Incidence of V3 descendants at forebrain level, though significant, is less than in more posterior parts, presumably reflecting construction of this pattern part from a more outlying region of the normal V3 domain. The grey background is formaldehyde-induced yolk fluorescence. **C, D**, Positive images of fluorescence in transverse sections at spinal cord (**C**) and hindbrain (**D**) level of second nervous systems. A neurite is visible in (**C**), and the characteristic appearance of neurogenic epithelium shows up in (**D**). E.v., ear vesicle; l., lumen of neural tube; n.p., neuronal cell extension; O.v., optic vesicle.

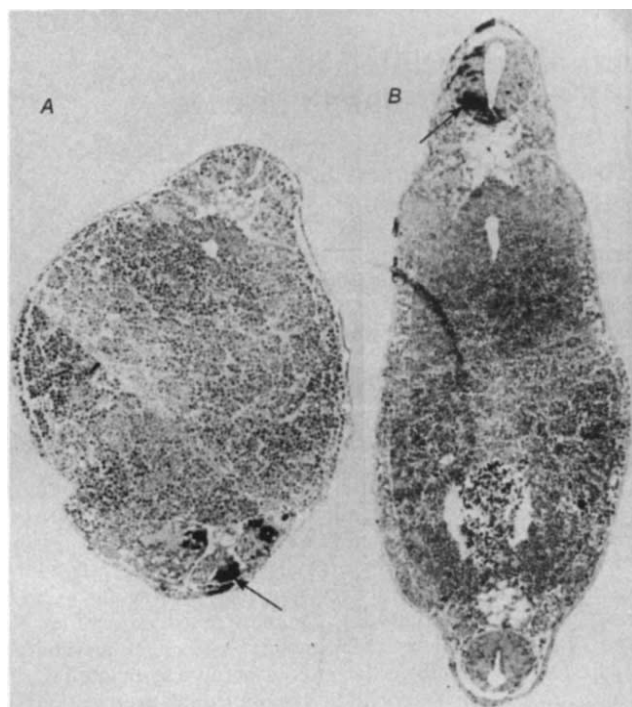


Fig. 4 Transverse sections of (**A**) V3-filled and (**B**) D2-filled experimental. **A**, Negative image of section at spinal cord level shows V3 descendants in epidermis, neural tube, and somites of the second axis, while the host neural tube is devoid of labelled (black) cells. **B**, Section at the anterior spinal cord level shows D2 descendants in epidermis, neural crest, and neural tube of the host axis, and no label in the second neural tube.

to form the host CNS did not migrate across the embryo to participate in the formation of the second neural axis. Without exception among our experimentally induced twin patterns, descendants of the originally labelled blastomere pair were found in one CNS, but not in both (Fig. 4).

Other work confirms that the CNS induced by small organizer grafts is substantially or wholly contributed by the host^{1,7,12}. The origin of these host cells has not, however, previously been identified. The grafted dorsal lip is thought to act by controlling the polarity of the pattern in adjacent mesoderm to give a new 'dorsal' midline, in relation to which the second CNS arises^{1,2,13}. Jacobson has recently marked *in situ* clones of cells from early embryonic stages in *Xenopus*, and visualized their final contributions to the anatomy in normal development⁴⁻⁶. It is claimed that all founder cells for the CNS are set aside within seven specific dorsally situated compartments, that include some 20 cells each, at around the ninth cleavage (512 cell blastula) long before neural induction is traditionally believed to occur. Jacobson proposed⁵ that, since such compartmentation had taken place, second CNS patterns induced at subsequent stages would prove to utilize cells migrating round the embryo from the appropriate origins elsewhere, involving substantial redistribution of cells within the neur ectodermal sheet.

The present results show clearly that second nervous systems, sometimes including each of the regions for which compartmental status has been proposed, are made by ectodermal tissue which happens to lie in appropriate spatial relationship to the graft. This tissue would normally have become belly and tail epidermis, and its development cannot have been diverted from this fate until well into gastrulation (some five or more cell generations after the 512 cell blastula), since the new stimulus is introduced only at the onset of that stage. This corresponds with the original conclusions of Spemann and his co-workers⁷.

Founder cell groups marked out in the blastula could conceivably be fundamental to normal CNS development, but since

cell interactions can later establish the same pattern among quite different cells, without any intervening regeneration or special growth of the responding tissue, we find this most unlikely. The data offered as demonstrating the particular early restrictions in question, in normal frog development⁶, in fact fall far short of those that establish the phenomenon in normal insect disc or blastoderm development³. It remains more reasonable to assume that the normal CNS is founded not much earlier than the extra CNS that can be induced, and by a similar mechanism, namely the imposition of a pattern of specifications upon a large population of pluripotent cells, only shortly before onset of the activities that then construct the organs. Genuine compartmentation may well prove to operate, however, in later episodes of vertebrate pattern formation.

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Scrapie-associated fibrils in Creutzfeldt-Jakob disease

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Scrapie of sheep, and Creutzfeldt-Jakob disease (CJD) of man belong to a group of transmissible encephalopathies which have been successfully transmitted to a variety of hosts¹⁻⁹. In susceptible hosts, these diseases are characterized by progressive degeneration of the central nervous system leading inevitably to death. The agents responsible for these diseases have not yet been identified, but they exhibit similar physicochemical characteristics. Abnormal fibrils designated 'scrapie associated fibrils' (SAF) have been observed in synaptosomal preparations of scrapie infected brain. They have never been observed in various types of control animals¹⁰⁻¹². We report here that SAF are present in CJD brain fractions in the experimentally transmitted disease as well as in a few naturally occurring human cases of CJD. SAF are also present in spleen extracts of animals experimentally infected with scrapie or CJD. This close associ-

ation of SAF with these two diseases and two different tissues (brain and spleen) known to contain titres of infectivity, suggest that the SAF are: (1) a unique pathological response to the disease or (2) the infectious agent of these diseases.

Low numbers of SAF first appear in scrapie infected mice about halfway through the incubation period and the number of fibrils increases steadily throughout the remainder of the preclinical and clinical phase of the disease^{11,13}. This pattern of increase is very similar to that of scrapie infectivity¹⁴. SAF have been observed in every strain of scrapie examined thus far (139A, ME7, 22A, 87V, 79A and 22L in mice, and strain 263K in hamsters). In contrast, SAF have not been detected in individual preparations prepared from over 75 control brains.

In three separate experiments CJD affected and control brains were fractionated and examined for SAF. Crude synaptosomal-mitochondrial fractions were prepared from homogenates of frozen brain, treated with octyl-glucoside and subjected to discontinuous sucrose density gradient centrifugation (method 4 in ref. 10). Note that crude synaptosomal-mitochondrial fractions in CJD contain the highest number of infectious units¹⁵⁻¹⁷. Fractions from the sucrose gradient were analysed by electron microscopy (EM) after negative staining, as previously described for scrapie¹⁰. The samples included the following: (1) Frozen cerebral cortex tissue from two different cases of human CNS disease; one of these was CJD as confirmed by neuropathological diagnosis and positive transmission to guinea pigs⁷. The second case, from its history and neuropathology, was diagnosed as familial Gerstmann-Straussler syndrome (GSS), which may be a variant of CJD¹⁸. (2) Three human cerebral cortex samples without a history of central nervous system (CNS) disease or evidence of CNS lesions on neuropathological evaluation were used as control material. (3) One strain of experimentally propagated CJD was studied in three different species; brain tissue

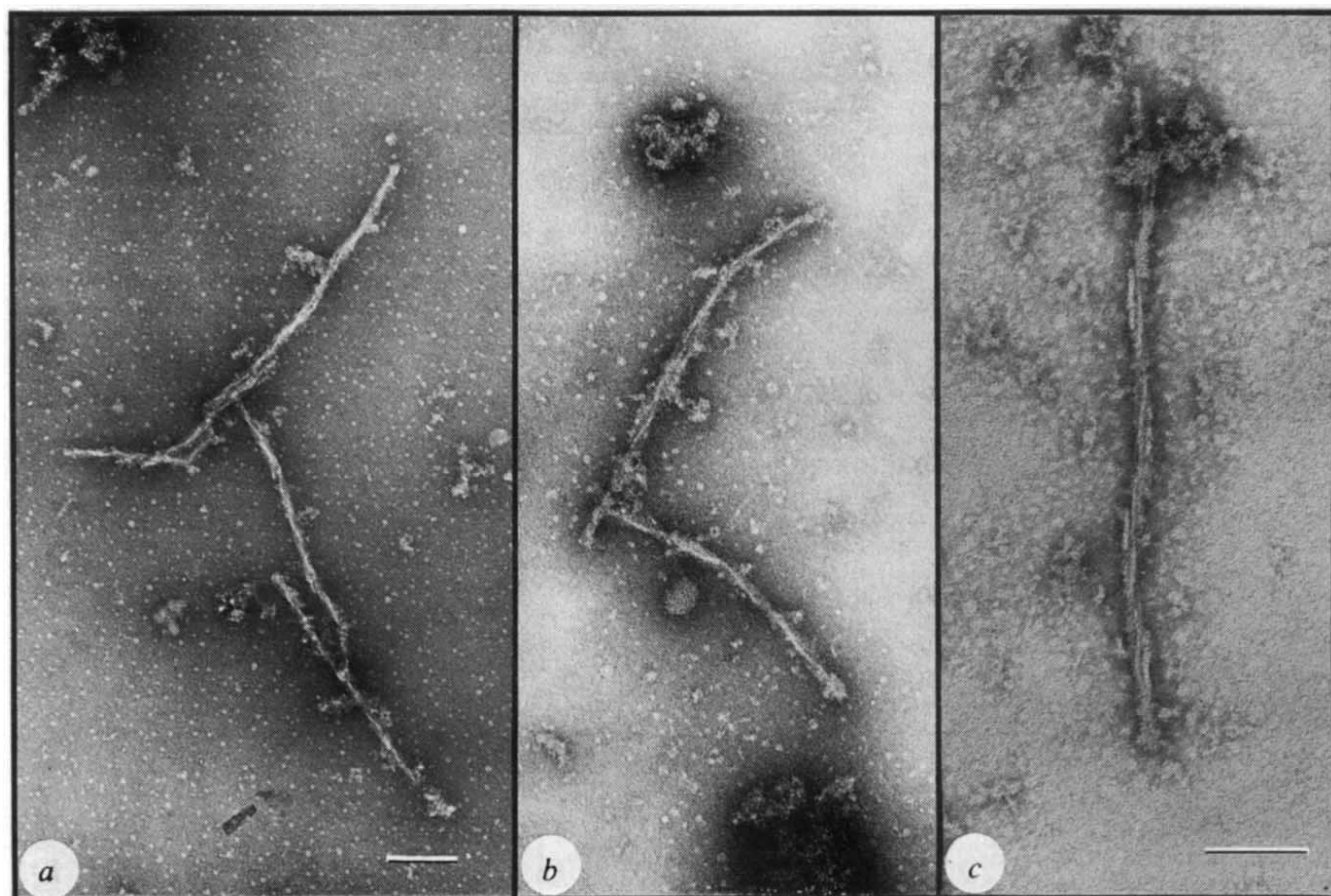


Fig. 1 Electron micrographs illustrating SAF structures observed in samples from *a*, CJD infected hamster brain, *b*, CJD human brain and *c*, 139A infected scrapie brain. All are stained with PTA. Magnifications: *a*, $\times 86,000$; *b*, *c*, $\times 133,000$. Scale bars, $0.1 \mu\text{m}$.

Table 1 Samples examined for the presence of SAF

Host species	Disease	Tissue	No. of samples	No. of samples containing SAF
Guinea pig	Control	Cerebral cortex	2	0
Guinea pig	CJD	Cerebral cortex	4	4
Hamster	Control	Cerebral cortex	2	0
Hamster	CJD	Cerebral cortex	4	4
Mouse	Control	Brain	2	0
Mouse	CJD	Brain	2	2
Human	Control	Cerebral cortex	3	0
Human	CJD	Cerebral cortex	1	1
Human	GSS	Cerebral cortex	1	1
Hamster	CJD	Spleen	1	1
Mouse	Control	Spleens (pooled)* (individual)	4 12	0 0
Mouse	Scrapie (139A)	Spleens (pooled)* (individual)	2 12	2 12
Mouse	Scrapie (ME7)	Spleens (pooled)* (individual)	3 1	3 1

Human tissue was removed aseptically at autopsy; portions of the brains were processed for histopathology and the remainder of the brains were frozen at -70°C until processed. Brains from guinea pigs, hamsters and mice, age matched or infected with CJD were removed aseptically and frozen at -70°C until processed. Brains and spleens were removed from C57BL/6J mice injected with normal mouse brain or scrapie strains 139A or ME7. The scrapie injected mice were in the clinical stage of the disease. The majority of the samples from the scrapie animals were processed immediately and a few of the samples were frozen at -70°C until fractionated. Results indicated that freezing the brains or spleens did not affect subsequent fractionation or electron microscopy. Cerebral cortex or whole brain (0.5–1.0g) was fractionated to obtain a crude synaptosomal mitochondrial pellet and overlaid on a discontinuous sucrose gradient as described previously (method 4; ref. 10). All fractionations were performed at 4°C . Frozen brain tissue was homogenized in a Dounce homogenizer with 25 strokes of a loose fitting pestle in 10 ml of solution A (0.32 M sucrose, 1 mM MgCl_2 , 0.5 mM KCl and 1 mM NaHCO_3). The homogenates were made 10% in solution A and centrifuged at 1,500g maximum for 10 min to separate the nuclei. The supernatant fluids were removed and reserved; the pellet was resuspended in 15 ml of solution A with a Vortex mixer and recentrifuged at 750g maximum for 10 min. The combined supernatants were centrifuged at 1,100g maximum for 10 min. The crude synaptosomal-mitochondrial fraction was separated from the supernatant by centrifugation at 17,000g max for 10 min. The pellet was resuspended in 4 ml of solution B (0.32 M sucrose, 1 mM NaHCO_3) by either mixing on a Vortex or by hand homogenization with a Dounce homogenizer. One ml of 5% octyl glucoside (octyl- β -D-glucopyranoside, Boehringer) in 100 mM Tris HCl pH 7.5, and 0.32 M sucrose was added. After a few minutes the solution cleared and was overlaid on a discontinuous sucrose gradient (5 ml of 1.8 M sucrose and 5 ml of 1.4 M sucrose in 100 mM Tris HCl pH 7.5). The gradients were spun at 85,000g maximum for 16 h in a Beckman SW 27.1 rotor and fractionated from the bottom in 1 ml fractions and the band at the interface between 1.4 M and 1.8 M sucrose was evaluated by electron microscopy. For spleen fractionation, spleens were homogenized in 1 ml of 0.2 M KCl with a motor driven Teflon tissue grinder. A further 9 ml of 0.2 M KCl was used in washing the homogenization tubes. The homogenates from the spleens were further processed as described above for the brain tissue. After the overnight spin on the discontinuous sucrose gradients, material was observable as a pellet and also at the interface between the 1.4 M and 1.8 M sucrose. Both these fractions were evaluated by electron microscopy. For electron microscopy, coded samples were diluted 1:4 or 1:10 with water and one drop applied for 1 min to freshly glow discharged carbon coated 400 mesh copper grids. Excess fluid was drained with filter paper and the sample stained for 1 min with 1 drop of phosphotungstic acid (PTA) pH 7.2 (adjusted with NaOH). The grid was air dried and examined in an RCA 3G electron microscope at 50 kV and $\times 11,000$ and on a Philips EM 300 at 80 kV at $\times 28,540$ through $\times 10$ binoculars. Routinely 10–20 squares of up to three grids for each sample were examined for the presence of SAF-like structures. Each grid was scored either: positive, at least one SAF clearly distinguishable; questionable, SAF probably present but not clearly distinguished from other material on the grid; or negative, no SAF-like structures detected. Information from all the grids prepared from each sample was collated to determine whether SAF were present or absent prior to the breaking of the code. All control samples were negative on all grids examined.

* 2–10 spleens were fractionated together for each determination.

from the first mouse passage⁹, from the first, fourth and fifth serial hamster passage⁸, and from the eighth and ninth serial guinea pig passage⁷ were sampled when all animals showed clinical signs of disease following inoculation with the CJD passaged strain. (4) Brains from uninoculated age-matched mice, guinea pigs and hamsters were used as control preparations (six samples). (5) Spleens, from both scrapie affected (strain 139A or ME7) and normal brain inoculated C57BL/6J mice were examined for the presence of SAF, since scrapie spleens contain infectivity (14); similarly one spleen from a CJD infected hamster (fifth passage) was also examined as spleens from CJD animals are also infective (ref. 15, and unpublished observations of L.M. and E.E.M.).

Coded specimens were analysed for SAF. The results are summarized in Table 1. Only human CJD and GSS brains and CJD infected animals contained SAF. SAF were observed in all spleens from all scrapie animals and the one spleen from a CJD affected animal. No SAF were found in control material. Examples of SAF from these experiments are shown in Fig. 1. SAF observed in a brain preparation from a scrapie strain 139A infected C57BL6J mouse are shown for comparison. Ultrastructurally SAF appear as short, straight, unbranched fibrils. They are dispersed freely as single fibrils, or in clusters, sometimes associated with proteinaceous or membrane like structures. Two types of SAF have been seen in a variety of preparations from scrapie infected animals. Type I SAF are composed of two 4–6 nm diameter filaments. Each of these two filaments is apparently helically wound around the other every 40–80 nm, yielding a 11–14 nm diameter fibre. The length of the fibres range from 100 to 500 nm. Type II SAF, observed in 139A and 263K infected animals, are composed of four filaments, again

each of 4–6 nm. The four filaments are also helically wound, but with a periodicity of 100–120 nm¹⁰. The morphology of the SAF in naturally occurring, or in experimentally transmitted CJD disease, was similar to that observed in scrapie, but primarily of SAF type I. SAF are a common feature in both diseases, and one more indication that these diseases are closely linked^{8,9}.

SAF are distinct from various other filamentous structures derived from brain. Our studies have shown that they differ substantially from a range of normal filaments of the brain including actin, microtubules, astrocytic filaments and neurofilaments. The SAF most closely resembled the published descriptions of amyloid fibrils. However, size and periodicity of the SAF were distinguishable from amyloid fibres isolated from various diseases and examined by us. SAF may be readily distinguished from the pathological paired helical filaments of neurofibrillary tangles^{11,12,19}. Other control preparations used for comparison have included nucleocapsids of measles and vesicular stomatitis virus, hepatitis B virus, and Fd phage. None of these showed any resemblance to SAF. In each case straining of these control preparations for electron microscopy was done under the same conditions as those for SAF¹¹.

One possibility is that SAF represent a unique pathological feature associated with the unrelenting course of these diseases in the brain. Since they have not been seen in aged brain samples or other natural²⁰ or induced diseases, they do not appear to be related to brain degeneration *per se*.

Furthermore, there are no reported histopathological changes in spleens of CJD or scrapie infected animals. Hence, the consistent presence of SAF in infected spleens (as well as in infected brains) raises an important alternative possibility, namely that

the SAF represent the infectious agents of scrapie and CJD. The evidence clearly shows that SAF are seen in all brains and spleens of all infected species examined thus far, regardless of the strain of the agent. Infectivity is known to be present in spleens. It is also present in partially purified SAF fractions from brain (R.A.S., manuscripts in preparation). Further purification of SAF should clearly show whether or not SAF are the infectious agent.

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Scrapie infectivity, fibrils and low molecular weight protein

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The development of a short incubation model of scrapie (strain 263K), in golden hamsters^{1,2}, has added impetus to the purification of the infectious agent. Our own attempts³ have been based on methods pioneered by Millson^{4,5} and developed by Prusiner^{6,7}. We present here results indicating that a purification factor of up to 10^4 with respect to protein may now be possible. Fractions from brain with high infectivity had a sedimentation range of 70-300S and contained an abundance of fibrils closely similar to the scrapie-associated fibrils (SAF) discovered by Merz *et al.*^{8,9}. Material of molecular weight (M_r) 26,000, which is probably protein, appears to be a major constituent of the fibrils. The association between infectivity and fibrils raises two possibilities: the fibrils are an infectious form of the scrapie agent or they are a pathological response to scrapie infection.

In previous work³ a relatively simple method was devised for the purification of scrapie infectivity (263K strain) from clinically affected hamster brain. It involves a series of differential centrifugation steps and the use of enzymes (proteinase K and

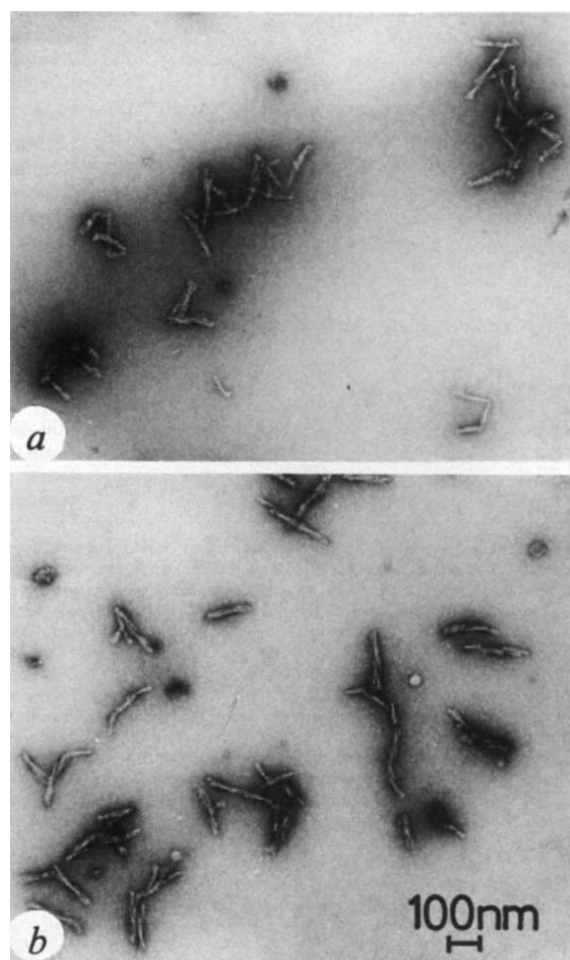


Fig. 1 Electron micrographs of samples of fractions 6 from the second sucrose gradient (see Table 1 legend) of scrapie-diseased CLAC hamsters (a) and AURA (b) hamsters. Samples were adsorbed to carbon-coated grids and negatively stained with 1% aqueous uranyl acetate¹⁵. The corresponding SDS-polyacrylamide gel electrophoresis protein patterns are shown in Fig. 2.

micrococcal nuclease) and sarcosinate to reduce contamination and render infectivity nonsedimentable at 22,000g for 40 min but sedimentable at 215,000g for 2 h. A final centrifugation at 215,000g for 2.5 h from 10% NaCl and 0.1% sarcosinate gave a pellet (designated P_{215S}) which represented about a 100-1000-fold purification of infectivity on the basis of protein³. Electron microscopic (EM) examination of the P_{215S} fraction by negative stain revealed fibrils³ like the SAF described by Merz *et al.*^{8,9} in crude brain extracts from scrapie-infected animals. In the present study we have obtained a further purification of infectivity and found that the improved methods also purify the fibrils to a high degree.

Samples of P_{215S} from the brains of control hamsters and hamsters with clinical scrapie were resuspended in buffer containing 1% sulphobetaine 3-14 (S3-14) and centrifuged on a sucrose density gradient containing 0.1% S3-14. The sedimentation markers (see Table 1 legend) indicated that material greater than 70S was in the pellet¹⁰. At least 80% of the recovered infectivity was in the pellet¹⁰ but only about 20% of the protein (data not shown). Hence, this first gradient removed some low molecular weight and low density contaminants, thus slightly increasing the specific scrapie titre of the major fraction.

After removing the sucrose, this fraction was centrifuged on a second sucrose gradient, identical to the first, but at reduced speed and time. This combination of successive gradient sedimentations means that the second one fractionated largely on the basis of size. Eleven fractions were collected and were

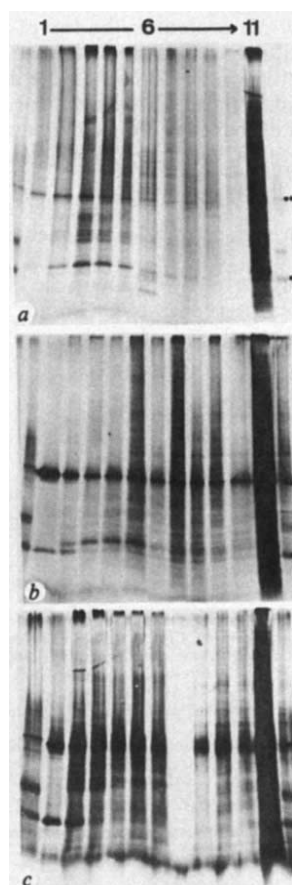


Fig. 2 SDS-polyacrylamide gel electrophoresis patterns of fractions collected from the second sucrose density gradient (see Table 1 legend). The figure shows the analysis of fractions 1–11 from CLAC hamsters, control (a) and scrapie (b), and from AURA scrapie hamsters (c). Most of sample from fraction 7c was lost, giving a near blank gel. Three standard proteins were run in identical conditions as shown in the outermost slots next to fractions 1 and 11; •, cytochrome *c* (M_r 12,300); unmarked band, β -lactoglobulin, (M_r 18,400); ••, α -chymotrypsinogen (M_r 25,700). After diluting to 10 ml, the fractions were centrifuged for 3 h at 200,000g. The pellets (visible only in fraction 11) were dissolved in 20 μ l of Laemmli buffer¹⁶ at 100 °C for 5 min and analysed *in toto* on linear, 12–17% polyacrylamide gels which were electrophoresed and then stained with silver¹⁷.

examined for scrapie infectivity, and by EM and polyacrylamide gel electrophoresis.

Table 1 shows that high amounts of infectivity (shortest incubation periods) were present in all fractions except fraction 1 at the top of the gradient. From the positions of the sedimentation markers it is clear that infectivity covered a range of 70–300S mainly, a range which includes small cubic animal viruses (parvovirus, picornavirus, papovavirus) and also rod-shaped plant viruses such as tobacco mosaic virus ($S = 190$).

EM observations showed that fractions of the gradient with high scrapie infectivity, and especially fractions 3–9, also contained large numbers of fibrils (Table 1). These accounted for at least 90% of all the visible structures (Fig. 1). Fibrils were rare in fraction 1, where infectivity was lowest, and they were absent from all the control fractions. At high magnification the fibrils appeared as short rods made up mainly of two helically twisted filaments, each of 4–6 nm diameter. By these criteria, they appear to be identical to the SAF discovered by Patricia Merz and her colleagues^{8,9} in brain extracts from scrapie animals. An EM grid of our fibrils has been sent to P. Merz who supports their identification as SAF. However, our fibrils

Table 1 Distribution of infectivity (measured by incubation period) and fibrils after sedimenting the CLAC scrapie preparation on the second sucrose gradient

Fraction no.	Sedimentation markers	Incubation periods (days $\pm$ s.d.)	Occurrence of fibrils
1 (Top)	50S	101 $\pm$ 2	Rare
2	70S	93 $\pm$ 5	Few
3		86 $\pm$ 0	Many
4	115S	91 $\pm$ 5	Many
5		82 $\pm$ 6	Many
6		92 $\pm$ 5	Many
7		91 $\pm$ 1	Many
8		87 $\pm$ 2	Many
9		87 $\pm$ 2	Many
10		93 $\pm$ 3	Few
11 (Pellet)		94 $\pm$ 2	Aggregates

P_{215S} pellets were prepared³ from pools of five frozen brains from each of three groups of hamsters: normal inbred CLAC hamsters, and CLAC and AURA hamsters with clinical scrapie (strain 263K). Each pellet was resuspended by sonication (3 bursts for 5 s) in 0.5 ml of detergent buffer (0.01 M Tris-HCl pH = 7, 4, 10% NaCl, 1% S3-14) which was layered on top of a 9.5-ml, 5–20% linear sucrose gradient containing 0.1% S3-14. Centrifugation was at 4 °C for 6 h at 200,000g in an SW41 rotor¹⁰. The pellets obtained were washed to remove sucrose by resuspending in 10 ml of detergent buffer (this time with 0.1% S3-14) and centrifuging for 3 h at 200,000g. The washed pellets were resuspended in 0.3 ml of detergent-buffer, kept at room temperature overnight, sonicated and centrifuged on a second gradient identical to the first but for only 90 min at 155,000g. At both gradient centrifugation steps, separate tubes were included containing bovine serum albumin (4–5S), *Escherichia coli* ribosomal subunits (30S and 50S courtesy of Professor H. Wittmann) and Φ X174 phage particles (empty 70S and full 115S). These reference standards were run in conditions identical to those used for the brain samples. After centrifugation, the reference standards were analysed by a continuous gradient collector equipped with an UV recorder. The brain-containing fractions were collected by needle puncture at measured distances down the side of each tube. Fractions 1–10 contained about 1.0 ml and for fraction 11, the pellet was made up to 1.0 ml with buffer alone. All fractions were sonicated. From each, 50 μ l were withdrawn for electron microscopy, 50 μ l taken for infectivity assay (CLAC samples only) and the remainder was diluted to 10 ml and sedimented as described for the pellet of the first gradient. The pellets were used for protein analysis (Fig. 2). To assay infectivity, the fractions were diluted with 1.0 ml of phosphate-buffered saline (giving a dilution of original brain of about 10^{-2}) and 50 μ l were injected intracerebrally into groups of three CLAC hamsters, all of which developed scrapie. The relative amounts of infectivity were measured by comparing the average incubation period produced by each fraction: the greater the infectivity, the shorter the incubation period. A difference of about 12 days between incubation periods reflects a 10-fold difference in infectivity³. The fibrils were observed by electron microscopy (see Fig. 1 legend).

were somewhat shorter (50–300 nm) than she observed (100–500 nm). This is probably due to the repeated sonication steps in the purification method we used. In the gradient there appeared to be an increasing length of fibrils from top to bottom (as might be expected), with dense aggregates in the pellet.

SDS-polyacrylamide gel electrophoresis of gradient fractions revealed a large number of bands, some of which varied in intensity between control and scrapie gradients, between different positions on each gradient (Fig. 2) and between different experiments. However, there were two major and consistent findings. First, most of the silver-stained material recovered from the gradients (say 90%) sedimented to the bottom. Hence, the two density gradient centrifugation steps probably increased the specific scrapie infectivities from 10^2 – 10^3 (P_{215S} starting material) to 10^3 – 10^4 (middle 60% of the second gradient). However, this enhanced purification must be confirmed by direct measurements of protein and by full titrations of infectivity. Second, the most striking feature of the scrapie fractions 1–10 was the predominance of a band whose migration coincided with that of α -chymotrypsinogen, indicating a molecular weight

of about 26,000 (Fig. 2). As the EM observations revealed a similar predominance of fibrils in most of these fractions (Table 1), we suggest that material in the 26,000- M_r band, presumably protein, is the major constituent of the fibrils.

Thus, we have demonstrated a method for purifying SAF and found an association between infectivity and the occurrence of fibrils. A similar association has been noted in crude extracts of brain taken from infected hamsters (263K strain of agent) or mice (139A strain of agent) during scrapie incubation: the numbers of SAF increased with agent titres⁹. Other studies on the purification of scrapie agent have revealed an association between infectivity and a protein of M_r 27,000–30,000, though not with the presence of fibrils^{11,12}.

There are two explanations for these findings. First, agent may cause the formation of fibrils as part of the disease process, and may fortuitously co-purify with them. The occurrence of SAF is one of the most consistent findings in scrapie^{8,9} (and in Creutzfeldt-Jakob disease¹³ which is similar). SAF also bear a structural resemblance to the fibrils of cerebral amyloid^{8,9}. In some scrapie models (depending on the strain of the agent and genotype of the host)¹⁴, cerebral amyloid plaques are a major pathological feature. Conceivably, the 26,000- M_r material may form SAF within cells (although they have not been identified in tissue sections) and sometimes form deposits of cerebral amyloid outside cells.

The second explanation is that the fibrils may represent an infectious form of the scrapie agent. This possibility is supported by the fact that fibrils have been found in extracts of scrapie-infected spleens as well as brains¹³. In our studies, fibrils were the only structure to be associated with relatively pure fractions containing high scrapie infectivity. The presence of large

amounts of 26,000- M_r protein at the top of the gradient (Fig. 2b, c) in which fibrils were rare and infectivity was lowest, suggests that infectivity may depend on the degree of aggregation of protein and the size of the fibril formed. Variation in fibril length could account for the sedimentation of infectivity in the range of 70–300S, with large aggregates of fibrils pelleting at the bottom.

Further purification of the fibrils and determination of their chemical structure will provide direct means of testing these hypotheses.

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Varicella-zoster virus DNA in human sensory ganglia

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Varicella-zoster virus (VZV) causes chickenpox and shingles¹. Clinical and epidemiological evidence indicates that following an episode of childhood chickenpox (varicella), VZV becomes latent, presumably in dorsal root ganglia, and is reactivated many years later to produce shingles (zoster) in adults². VZV has been demonstrated in ganglia by electron microscopy and by indirect immunofluorescence^{3–5}, and infectious viral particles have been isolated from acutely infected ganglia of patients who died of disseminated VZV infection⁶. However, VZV has not been detected in the ganglia of humans without recent exposure to VZV. Tissue culture explant methods that have been successful in the isolation of herpes simplex virus from ganglia have so far failed in the isolation or reactivation of VZV from trigeminal and other dorsal root ganglia⁷. We describe here the detection of VZV DNA sequences in an acutely infected human sacral ganglion and in normal trigeminal ganglia. These findings support the hypothesis that VZV is latent in normal human ganglia.

VZV DNA was cleaved with the restriction endonuclease *Bam*HI and DNA fragments were cloned in the plasmid vector pBR322. Seven recombinant clones consisting of nine *Bam*HI-cleaved VZV DNA fragments (46% of VZV DNA with a size of 125 kilobase pairs, kbp) were isolated and their locations on

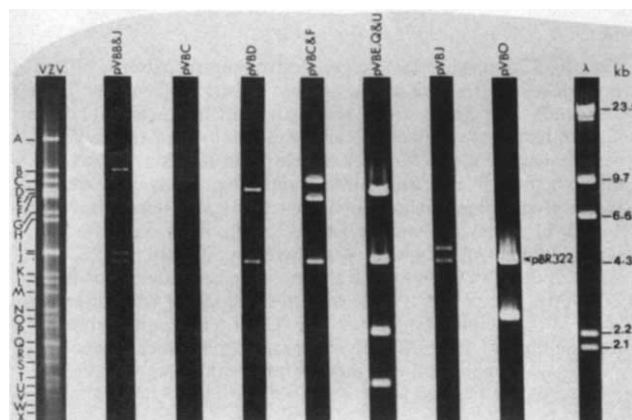


Fig. 1 Agarose gel electrophoresis of *Bam*HI-digested varicella-zoster virus (VZV) DNA and cloned DNA fragments. Viral DNA was extracted from purified virions and from VZV-infected cells as described elsewhere^{9–11}. VZV DNA was cleaved with the restriction endonuclease *Bam*HI and the DNA fragments were cloned in the plasmid vector pBR322 as described previously¹². VZV and recombinant DNAs were digested with *Bam*HI and DNA fragments were separated in 0.6% agarose gels. The recombinant DNAs were designated by four letters according to Ecker and Hyman⁸: p, plasmid pBR322; V, VZV; B, *Bam*HI; and the letter of cloned *Bam*HI fragment(s). The left lane (VZV) shows the pattern of *Bam*HI-cleaved VZV DNA fragments. *Hind*III-cleaved λ DNA fragments (right lane) were used as size markers.

the physical map were determined as shown in Figs 1–3. Cloned DNA fragments were labelled with ³²P and hybridized to VZV DNA cleaved with *Bam*HI and *Hind*III. VZV DNA end fragments were determined by hybridization of ³²P-labelled cloned DNA fragments to VZV DNA digested with exonuclease. VZV DNA (1 μ g) was digested with 100 units of exonuclease III in 20 μ l of 50 mM Tris-HCl, pH 8.0, 10 mM 2-mercaptoethanol and 5 mM MgCl₂ for 45 min at 37°C. The reactions were

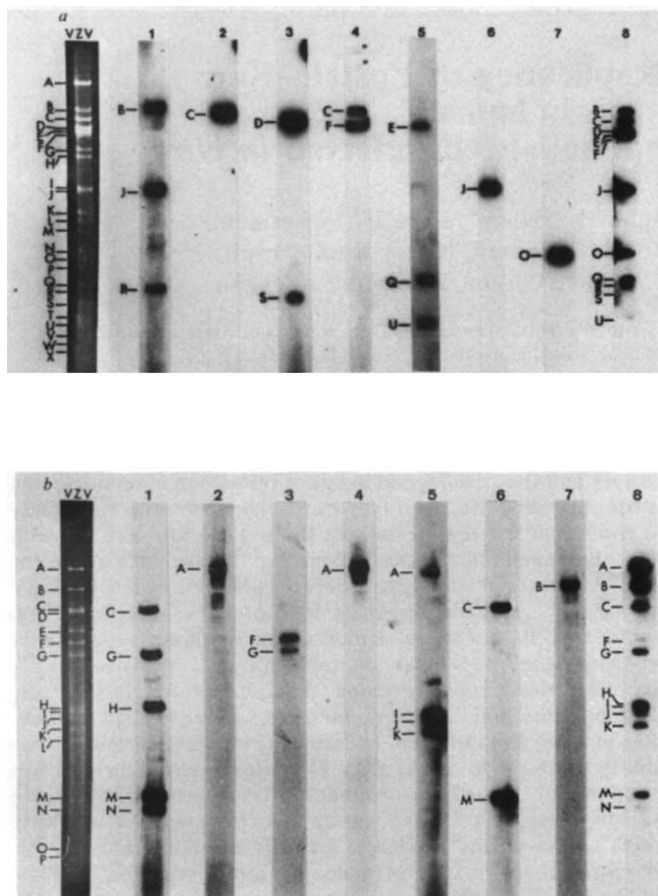


Fig. 2 Hybridization of VZV DNA cloned fragments to VZV DNA cleaved with *Bam*HI (a) and *Hind*III (b). DNA fragments were separated in 0.6% agarose gels and blot-transferred to nitrocellulose filters as described previously^{13,14}. Individual strips were cut and hybridized to nick-translated ³²P-labelled VZV cloned DNA fragment(s) as described elsewhere¹⁵. Left lanes (VZV) show the cleavage pattern of VZV DNA digested with *Bam*HI (a) and *Hind*III (b). The probes for a and b were pVBB and J DNA (lane 1), pVBC DNA (lane 2), pVBD DNA (lane 3), pVBC and F DNA (lane 4), pVBE, Q and U DNA (lane 5), pVBJ DNA (lane 6) and pVBO DNA (lane 7). Lane 8 shows hybridization of pooled VZV DNA cloned fragments to VZV DNA cleaved with *Bam*HI (a) and *Hind*III (b). Hybridization of pVBB and J DNA to *Bam*HI B, J and R fragments demonstrates that the terminal repeated sequences (*Bam*HI R) are repeated internally (*Bam*HI B) as shown in Fig. 3.

stopped by heating at 65 °C for 15 min and cooling at 4 °C. The DNAs were then digested with either *Bam*HI, *Hind*III or *Sal*I for 3 h at 37 °C, and the DNA fragments were separated in 0.6% agarose gels, transferred to nitrocellulose filters and hybridized to ³²P-labelled cloned VZV DNA fragments. The results (not shown) were in agreement with Ecker and Hyman⁸ and indicated that the cloned *Bam*HI E fragment is located at the near left-hand end of VZV DNA and that the cloned *Bam*HI B and J fragments contain terminal and inverted repeat sequences.

To detect VZV DNA sequences in human sensory ganglia, cloned DNA fragments were labelled with ³²P and hybridized to *Bam*HI-cleaved DNA from human ganglia. A sacral ganglion was removed from an 82-yr-old man who developed sacral distribution zoster 3 days before death. VZV was isolated from his spinal fluid and at autopsy, the sacral ganglion revealed haemorrhagic necrosis and Cowdry A inclusion bodies. Trigeminal ganglia were removed from 10 humans who died from drowning (1), peritonitis (1), gunshot wounds (3), multiple sclerosis (2), congenital heart disease (1), olivopontocerebellar degeneration (1) and a degenerative encephalopathy of unknown aetiology (1). Two VZV DNA cloned fragments (pVBC and pVBD) which do not share sequence homology with normal human brain cell DNA, normal liver cell DNA and monkey kidney cell DNA (in which VZV was propagated), or with DNA of herpes simplex virus, cytomegalovirus and Epstein-Barr virus were selected for the detection of VZV DNA sequences in human sensory ganglia. pVBC (0.16–0.24 map units) and pVBD (0.69–0.79 map units) were labelled with ³²P (specific activity 2–5 × 10⁸ c.p.m. per µg DNA) and hybridized to DNA extracted from ganglia. Hybridization of ³²P-labelled cloned DNA fragments (pVBC and pVBD) to DNA from a trigeminal ganglion from a 53-yr-old male who drowned resulted in the detection of VZV DNA sequences as shown in Fig. 4. Hybridization of seven clones (nine *Bam*HI fragments) to DNA from the sacral ganglion resulted in the detection of all the cloned fragments, indicating the presence of the complete VZV genome in the sacral ganglion (Fig. 4). In addition, hybridization of pVBC to DNA from 13 trigeminal ganglia from 9 humans also resulted in the detection of VZV DNA sequences in 5 ganglia from 3 humans (results not shown). The five positive ganglia were from a 29-yr-old male who died from a gunshot wound (2/2), a 50-yr-old male who died from a gunshot wound (1/2) and an 8-yr-old boy who died from a degenerative encephalopathy of unknown aetiology (2/2). No VZV DNA sequences were found in DNA from one human brain or 16 human livers. Nick-translated ³²P-labelled pBR322 alone did not hybridize to DNAs from human ganglia, normal human brain, normal human liver or monkey kidney cell (results not shown).

Densitometric quantitation (taken from autoradiograms) from DNA bands of known concentrations of DNA cloned

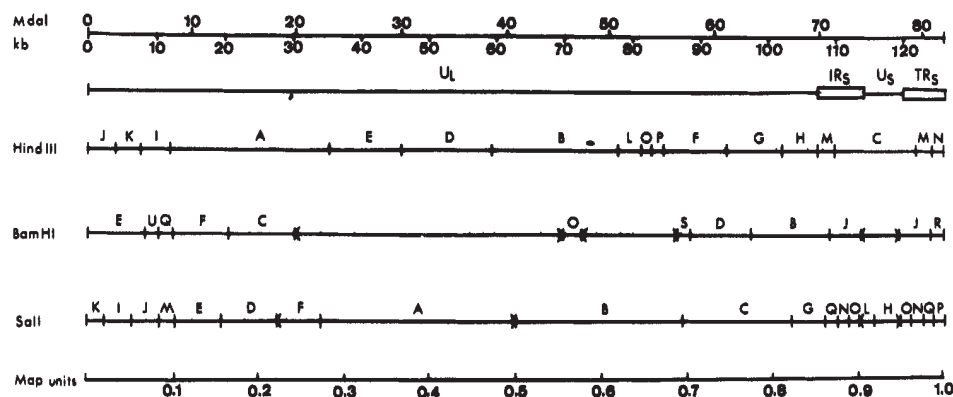


Fig. 3 Physical map of VZV DNA cleaved with the restriction endonucleases *Bam*HI and *Sal*I. The map of *Hind*III-cleaved VZV DNA has been determined by Ecker and Hyman⁸. Letters represent individual fragments produced by the restriction endonucleases (Fig. 2). DNA end fragments were determined by hybridization of VZV DNA cloned fragments to DNA digested with exonuclease III. UL, unique sequences of long DNA segment; US, unique sequences of short DNA segment; IRs, internal repeat sequences; TRs, terminal repeat sequences. Mdal (megadaltons), kbp (kilobase pairs). The relative order of *Bam*HI and *Sal*I fragments in parentheses have not been determined.

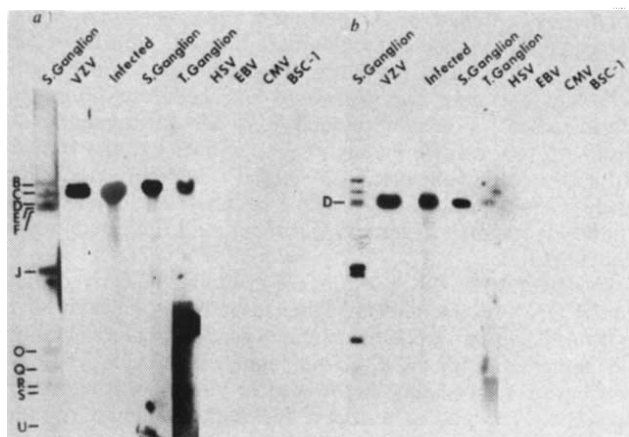


Fig. 4 Detection of VZV DNA sequences in human sensory ganglia. *Bam*HI-cleaved VZV DNA cloned fragments C and D (Fig. 1) were labelled with 32 P and hybridized to *Bam*HI-cleaved VZ virion DNA (0.5 μ g), DNAs from VZV-infected cells (0.5 μ g), sacral ganglion (20 μ g), trigeminal ganglion (20 μ g) and 0.5 μ g of DNAs from herpes simplex virus (HSV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) and 20 μ g of monkey kidney cell (BSC-1). The probe for *a* was pVBC and for *b* was pVBD. Right lanes in *a* and *b* show hybridization of pooled 32 P-labelled VZV DNA cloned fragments to DNA from the sacral ganglion of a man who had sacral distribution zoster just before death. The ganglia were Dounce-homogenized in TNE buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 50 mM EDTA) at 4 °C, digested with SDS (2% final concentration) and proteinase K (500 μ g ml $^{-1}$) for 18 h at 37 °C. The DNA was extracted twice with phenol and three times with chloroform, precipitated with ethanol and resuspended in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). All extracted DNAs were incubated with RNase (50 μ g ml $^{-1}$) for 2 h at 37 °C, phenol-chloroform extracted, precipitated with ethanol and dissolved in TE buffer. Approximately 300 μ g of DNA was obtained from the trigeminal ganglion, and 140 μ g from the sacral ganglion. HSV-DNA was a gift from Dr Nigel Fraser, CMV from Dr E-S Huang, and EBV from Dr Elliott Kieff.

fragment C (9.7 kbp), ganglion DNA (6×10^6 kbp) and VZV-infected cell DNA indicated the presence of approximately 0.28–1, 60 and 700 copies of DNA fragment C in trigeminal, sacral and infected cells, respectively, assuming the presence of one viral DNA molecule in each cell. The detection of 60 copies of viral DNA in the sacral ganglion of a patient with sacral distribution zoster just before death demonstrates the active replication of VZV in the ganglion. However, the presence of approximately one copy of VZV DNA in normal trigeminal ganglia of humans without clinical zoster supports the hypothesis that VZV remains dormant in human sensory ganglia.

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Note added in proof: After submission of this manuscript, the detection of VZV RNA in human trigeminal ganglia by *in situ* hybridization was also reported¹⁶.

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Replication of Epstein-Barr virus in human epithelial cells infected *in vitro*

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Epstein-Barr virus (EBV), a member of the herpes group of viruses and the aetiological agent of infectious mononucleosis, is usually thought of as a lymphotropic virus with the ability to transform B lymphocytes. So the association of EBV with nasopharyngeal carcinoma is puzzling^{1–4}, especially given the lack of success of attempts to infect epithelial cells with EBV in culture^{5,6} and the apparent lack of EBV receptors^{7,8} on epithelial cells^{5,6}. Circumvention of the apparent requirement for membrane receptors by techniques of transfection^{9,10}, microinjection¹¹ and receptor transplantation⁵ has clearly demonstrated that there is no inherent barrier to EBV replication in nonlymphoid cells, including epithelial cell types. Our ability routinely to detect EBV DNA by *in situ* hybridization in epithelial cells of the oropharynx from persons with acute infectious mononucleosis suggests that, *in vivo*, EBV regularly gains access to and replicates lytically in epithelial cells^{12,13}. We report here *in vitro* evidence for direct infection by EBV and replication of the virus in cultured normal human epithelial cells.

Primary explant cultures of human ectocervix obtained at hysterectomy were used in these experiments. A known target for other herpes-group viruses, cervical epithelium is free from gross inflammatory cell infiltration characteristic of surgical specimens from the nasopharynx. Pavement-like cell sheets grow out in a stratified pattern within 14 days of initiation of culture. Fifty to seventy-five coverslip explant cultures can be obtained from a single tissue donor; tissue from 15 donors was used for infection in these experiments. Careful dissection of stroma, preselection of batches of fetal calf serum (FCS) and growth in minimal essential medium containing D-valine (MEM D-Val, Gibco) result in cultures free from contaminating fibroblasts. The growth and epithelial characteristics of these cultures have been described elsewhere^{14,15}.

Virus from two sources was selected for purposes of infection: from concentrated supernatant fluid from the productively infected lymphoblastoid cell lines P3HR1, MCV-5 and B95-8; and from filtered oropharyngeal washings from nine patients with acute infectious mononucleosis. EBV-induced antigens were detected in epithelial cultures exposed to virus from throat washings by standard indirect immunofluorescent techniques with multiple human sera containing antibodies to viral capsid antigen (VCA), early antigen (EA) and VCA, and EBV nuclear antigen (EBNA) (Fig. 1*a–d*). Approximately 0.05% of cells in the infected cultures expressed EA, VCA or EBNA (Table 1). The number of EBV antigen-containing cells did not increase over a 30-day period of observation. Antigen-bearing cells were scattered throughout the cell layer with no foci of infection observed. EBV antigens could not be detected until after day 8 post-infection. Verification of these findings by *in situ* hybridization with an EBV-specific cRNA probe revealed a similar number of cells containing large amounts of EBV DNA (Fig. 1*e, f*). However, supernatant fluids from antigen-positive cell cultures have been without transforming activity for cord-blood lymphocytes. No morphological alterations or evidence of cell transformation were observed in epithelial cultures.

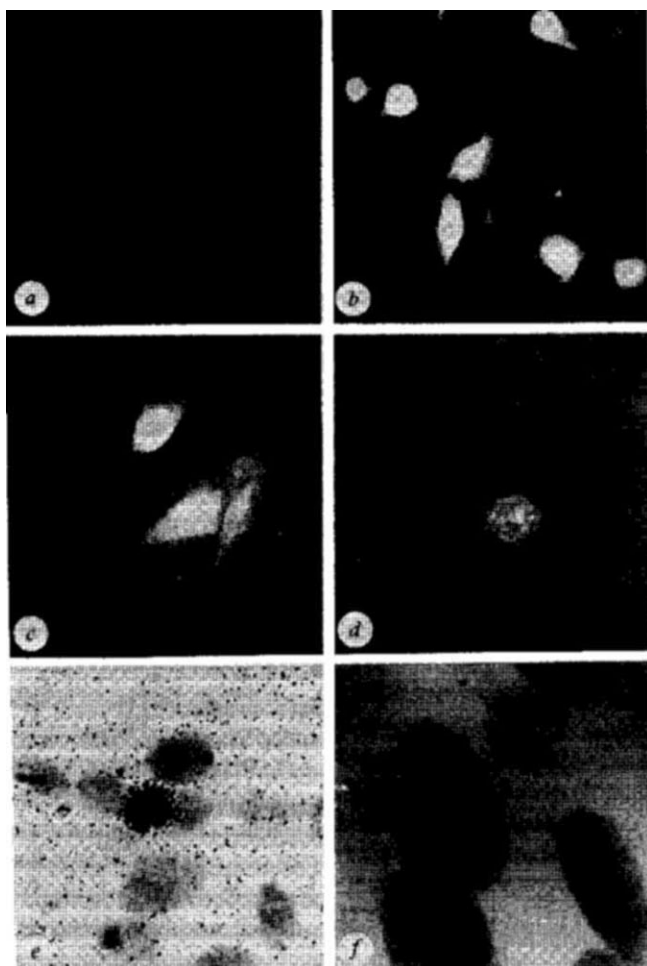


Fig. 1 EBV antigens and DNA in epithelial cell cultures infected *in vitro* with EBV from throat washings. Coverslip explant cultures for EA/VCA determination were fixed at -20°C in acetone for 10 min; for EBNA, 3 min in 1:1 methanol/acetone; for *in situ* hybridization, 15 min in 3:1 methanol/acetic acid. Staining for EA/VCA and EBNA was by standard indirect immunofluorescence²⁰ and anti-complement immunofluorescence²¹ respectively. ^3H -labelled cRNA probes for *in situ* hybridization were synthesized as described previously¹⁹ to the cloned EBV DNA *Bam*HI V fragment, an internally repeated sequence of the EBV genome, as well as to purified EBV DNA derived from P3HR1 lymphoid cell cultures. Before hybridization, DNA in infected epithelial cell cultures was denatured with 0.07 M NaOH for 3 min. $1-2 \times 10^5$ c.p.m. ^3H -cRNA were hybridized under coverslips for 22 h at 66°C . Slides were washed four times in $2 \times \text{SSC}$, treated for 30 min with $40 \mu\text{g ml}^{-1}$ RNase, washed successively in $2 \times \text{SSC}$, 70% and 95% ethanol, coated with Kodak Nuclear Tract NTB2 emulsion, dipped in Dioxane with 1% diphenyloxazole, 0.02% *p*-phenylene-phenyloxazole, and held at -70°C in a desiccating slide box for 6 days before developing. All hybridizations were performed with productively infected P3HR1 cells as a positive control¹⁹ and with latently infected Raji cells¹⁹ as well as mock-infected epithelial cell cultures as negative controls. *a*, Mock-infected epithelial cell layer stained for EA/VCA. *b*, *c*, VCA expression in epithelial cell layers infected with EBV from throat washing no. 3 of a patient with acute infectious mononucleosis (detected with 1:10 dilution of human antiserum, anti-VCA antibody titre 1:320, EA < 1:4). *d*, Cell layers stained for EBNA. Note granular pattern of nuclear fluorescence characteristic of EBNA. Nuclei of adjacent cells are negative. *e*, *f*, Autoradiographs of cRNA-DNA *in situ* hybridization of EBV-infected epithelial cell cultures using the two different probes described above. In *e*, nuclei of cells are stained prominently with methylene blue and grains are concentrated over an EBV DNA-containing cell. In *f*, note the superimposed nuclei characteristic of cell stratification and accumulation of grains over the nucleus of the more superficial cell.

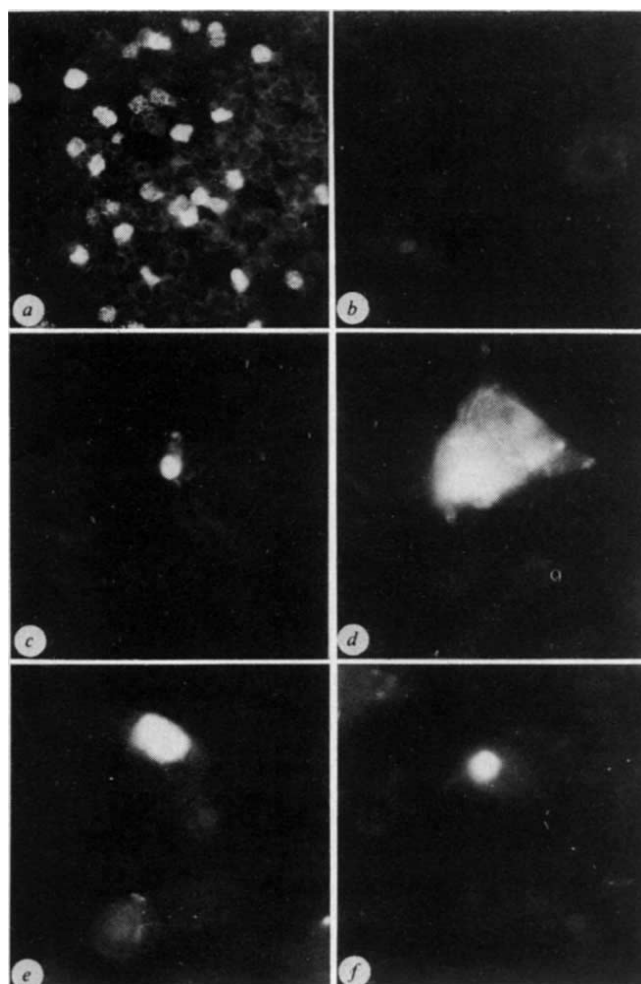


Fig. 2 EBV antigen and DNA-bearing epithelial cells sloughed from infected but antigen-negative cell layers. Shed cells were collected and fixed as described in Table 2 legend. A pool of anti-EA (restricted), anti-EA (diffuse) and anti-VCA mouse monoclonal antibodies (Biotech) at 1:100 dilution was used together with fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG at 1:20 dilution to detect antigens of EBV replicative cycle. EBV DNA-containing cells were identified by *in situ* hybridization with a biotinylated nick-translated *Bam*HI V cloned DNA probe prepared as described elsewhere²² (Enzo Bioprobe). The DNA probe was heat-denatured before application to cells. Slides were heated for 5 min at 80°C , then cooled to 37°C and incubated in a water bath for 22 h. FITC-labelled avidin (Vector Labs) at 1:500 dilution was used to detect hybridized biotinylated DNA. *a*, EA in superinfected Raji cell control detected with mouse monoclonal antibody pool. *b*, Epithelial cells shed from mock-infected epithelial cell cultures stained for EA/VCA. Note the large cytoplasmic to nuclear ratio characteristic of differentiated squamous epithelial cells. *c-e*, EA/VCA-bearing epithelial cells shed from virus-exposed cell layers. Most cells displayed a pattern of nuclear fluorescent but some cytoplasmic staining is seen as well. *f*, Shed epithelial cells with nuclear hybridization detected with biotinylated *Bam*HI V DNA probe specific for EBV DNA.

To ascertain whether cells exfoliating from infected epithelial cell cultures contained viral antigens, we collected cells from culture supernatant fluids shed over a 3-4-day period (Table 2). In two experiments, desquamated cells collected after day 9 post-infection contained VCA when tested with human antiserum (VCA 1:320, EA < 1:4). In a third study, despite the fact that viral antigens could not be detected in the epithelial cell sheet itself, we demonstrated antigens indicative of viral replication in exfoliated cells collected 12 days post-infection using a pool of mouse monoclonal antibodies to EA (diffuse and restricted components) and VCA (Fig. 2*c-e*). EBV DNA

Table 1 Evidence for infectivity of EBV oropharyngeal isolates for normal cultured human epithelial cells

Culture	Virus used Wild type from oropharynx Cord-blood transforming activity	EBV DNA in epithelial cells shed into TW	Positive cultures per number exposed*	Infectivity for cervical epithelium		
				Assays used		<i>In situ</i> hybridization
				EBNA	EA/VCA	
TW40	+	+	13/15	+(ND)	+(ND)	+(ND)
TW101	+	+	7/11	+(12)	+(45)	+(8)
TW1	+	+	2/4	ND	+(82)	+(12)
TW7	+	—	2/4	ND	+(43)	+(29)
TW3	+	ND	2/3	+(9)	+(15)	ND
TW pool	+	ND	3/3	ND	+(21)	+(17)
TW9	—	—	0/2	—	—	ND
TW4	—	ND	0/2	—	—	ND
TW GD	—	ND	0/20	—	—	—
TW BF	—	—	0/12	—	—	—
Laboratory strains						
P3HR1			0/14	—	—	—
MCUV-5			0/25	—	—	ND
P3HR1 + MCV-5			0/12	—	—	ND
B95-8			0/3	—	—	—

Throat washings (TWs) were obtained from patients with acute infectious mononucleosis by gargling 10 ml phosphate-buffered saline solution (PBS). Cells shed from the oropharynx were separated by centrifugation at 1,200 r.p.m. for 15 min, washed twice, fixed at -20°C in 3:1 methanol:acetic acid and dropped onto a -20°C glass slide for *in situ* hybridization with a ^3H -labelled cRNA probe to the cloned *Bam*HI V fragment of the EBV genome. The supernatant fluid was passed through a 0.22- μm filter, and the presence of EBV determined by standard cord-blood lymphocyte transformation assay¹⁸. TWs 1, 3, 9 and 4 were used as a source of virus on epithelial cultures and their infectivity for epithelium determined before documentation of the presence of infectious virus by cord-blood lymphocyte transformation assay. P3HR1, MCV-5 and B95-8 strains of EBV were prepared from respective cell lines as described elsewhere¹⁹, and concentrated 100–250-fold by centrifugation. Biological activity of virus preparations was ascertained by cord-blood lymphocyte transformation assays and superinfection of Raji cells. Virus stock was diluted 1:1, 1:10, 1:100 in PBS for infection. Epithelial cells grown to confluency (2 weeks) were washed twice and exposed for 2 h to 0.2 ml virus preparation. The inoculum was aspirated, fresh medium added and cultures incubated at 37°C in 5% CO_2 . Cell cultures were fed every 3–4 days with MEM D-Val (Gibco), 10% FCS, $5\text{ }\mu\text{g ml}^{-1}$ hydrocortisone, 2 mM glutamine and penicillin/streptomycin. Replicate coverslip cultures were fixed at each time interval and specific antigen determinations performed on separate coverslips. Coverslips to be stained were washed twice and fixed. Multiple human antisera at 1:10 dilution were used for EBNA (titre 1:160, VCA (titre 1:320) and EA/VCA (1:160/1:320) determinations. EBV-negative sera were used as controls on infected cells. Each assay was run with the following control slides: P3HR1 for VCA, superinfected Raji cells for EA, Raji for EBNA, P3HR1 and BJAB (or Raji) for *in situ* hybridization, and mock-infected epithelial cell culture for each separate antigen determination. Slides were read under blind code with Leitz immunofluorescence microscope; all fields, representing 10^5 cells, were examined under a $\times 26$ objective. 'TW pool' was from two patients with acute infectious mononucleosis. TW GD and TW BF were healthy seronegative controls. All remaining donors were EBV seropositive with a clinical syndrome of acute infectious mononucleosis. Numbers in parentheses represent number of positive cells scored in a representative experiment from confluent cell sheet of 10^5 cells. ND, not determined.

* Data reflect number of coverslips positive by all techniques versus total number tested. 15/15 virus-exposed cultures tested before day 9 were negative for EBV antigen and are not included in this summation.

was detected in sloughed cells by hybridization with both a ^3H -labelled EBV-specific cRNA probe and a biotinylated nick-translated *Bam*HI V DNA probe (Fig. 2f, Table 2).

Only epithelial cell cultures exposed to wild-type virus from fresh oropharyngeal washings were successfully infected. As in earlier reports^{5,6}, virus concentrates from culture supernatant fluids of established productively infected lymphoid cell lines failed to induce EBV antigens in primary epithelial cell cultures (Table 1). Moreover, all mock-infected cells lacked evidence of EBV infection by immunofluorescence or *in situ* hybridization techniques. Throat-washing samples from two EBV-seronegative persons as well as specimens from two patients with infectious mononucleosis, but without transforming activity on cord-blood lymphocytes, were unable to induce EBV antigens. Significantly, expression of EA/VCA was blocked in cultures exposed to throat washings preincubated with human serum containing EBV-neutralizing antibody, but not in those infected with the identical untreated virus.

Use of laboratory strains of EBV may explain earlier failures to infect cultures of human nasopharyngeal epithelium^{5,6}. EBV excretion in the saliva of both healthy and ill persons points to the existence in the oropharynx of a cell type permissive of virus replication. Virus collected directly from such a primary target cell should manifest optimal biological activity. All the laboratory strains have either arisen in or been long adapted to lymphoid cells. Our detection of EBV DNA in oropharyngeal epithelial cells from three of the four donors whose throat washing successfully initiated infection of epithelial explant

Table 2 EBV DNA and antigens of the virus replicative cycle in epithelial cells shed from cell-culture layer

Infecting virus	Day (D) post-infection cells collected in supernatant fluids		
	Positive or negative for EA/VCA	DNA present or absent	
TW3*	D6(—)	D11(+) [ND]	ND
TW101*	D6(—), D9(—)	D11(+), D13(+), D17(+) [10/1,000]	D14(+) [12/1,000]
TW pool†	D5(—)	D12(+) [7/1,000]	D14(+) [4/1,000]
TW pool (neutralized by antiserum)†	D5(—)	D12(—)	ND
Mock Infected*†	D5(—), D6(—)	D12(—), D13(—), D17(—)	D14(—)

Culture medium overlying cell layers for 3–4 days was aspirated, spun at 1,200 r.p.m. for 15 min and the cell pellets washed twice. Cells were spun onto glass slides using a Shandon Cytospin 2 at 950 r.p.m. for 7 min and fixed as for Fig. 1 for EA and VCA or for *in situ* hybridization. Each assay performed represents the pooled supernatant fluids from at least four replicate cultures; approximately 10^4 desquamated cells were obtained for study. Values in square brackets represent positive cells/total counted from a single assay. ND, not determined.

* VCA detected by human antiserum (1:10 dilution) with VCA titre of 1:320, EA <1:4. EBV DNA detected by ^3H -labelled EBV-specific cRNA probe to *Bam*HI V fragment.

† EA/VCA detected by pool of mouse monoclonal antibodies to diffuse plus restricted EA and VCA (1:100 dilution; Biotech). EBV DNA detected by biotinylated nick-translated *Bam*HI V DNA probe.

cultures (Table 1) implicates epithelial cells as the source of the EBV isolates used, a factor which might provide additional virulence.

The absence of foci of infection in cell layers suggests abortive infection or presence of virus receptors on only a few cells. In both cases, virus would not be expected to spread to neighbouring cells. Based on our detection of VCA as well as EBV DNA by hybridization in some cells, we conclude that a productive infection did occur. Failure to recover infectious virus from culture supernatant fluids may reflect the small number of cells actually infected and the insensitivity of the cord-blood assay.

The relation between EBNA synthesis and EA/VCA expression in this system requires further study. The delay in appearance of viral antigens, the impression that it was the cells in the uppermost layer of the cell sheet which contained large amounts of virus (Fig. 1f) and the ability in some instances to detect structural antigens and EBV DNA in desquamated but not attached cells all suggest that epithelial cells, once infected, become permissive of viral replication only on reaching a certain phase of cell maturation. Based on our frequent observation of EBNA in mitotic cells, it is tempting to speculate that EBV, like the Shope papilloma virus and Marek's disease virus, may be carried latently in rapidly dividing cells of the basal epithelium and actively replicate only on cell differentiation or senescence. The detection of large amounts of EBV DNA in exfoliated cells from the oropharynx of patients with infectious mononucleosis is consistent with this view^{12,13}.

Glaser and associates have reported EA expression in as many as 25% of epithelial cells from a primary explant culture of nasopharyngeal carcinoma after superinfection with P3HR1 virus¹⁶. These results were interpreted to suggest receptors do exist on a subset of nasopharyngeal carcinomas. Our findings in ectocervical tissue indicate that normal human epithelium harbours at least some cells with EBV receptors. Perhaps nasopharyngeal carcinoma represents a clonal expansion of this subset of cells with virus receptors. Receptor-positive cells may be less differentiated, and thereby protected from the lytic effects of the virus replicative cycle. If cell transformation occurs, the latently infected epithelial cell may represent the progenitor of nasopharyngeal carcinoma¹⁷.

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Unusual sequences in the murine immunoglobulin μ - δ heavy-chain region

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The δ heavy (H) chain of mouse immunoglobulin D (IgD) is unusual both in its structure and in its differential expression relative to immunoglobulin M (IgM; reviewed in ref. 1). The region of DNA between IgM and IgD H-chain constant-region genes is probably implicated in this control. So far only fragments of the area have been sequenced. Now, however, we present the complete sequence as well as the sequence of the introns of the C_δ gene. We have found several interesting features (Fig. 1), including an open reading frame (ORF) between C_μ and C_δ which encodes 146 amino acids that might represent a previously unsuspected domain-like protein; three blocks of simple repetitive sequences; a 162-base pair (bp) unique-sequence inverted repeat; and a domain-like pseudogene in the large intron of C_δ . We have not found, however, any sequence 5' of C_δ resembling the switch (S) recombination sequences associated with class switching in other heavy chains^{2–5}. Moreover, we have determined the 3' deletion end point of an IgD-producing myeloma and find no sequences reminiscent of switch sites nearby.

The DNA sequence from the end of C_μ 4 to the end of C_δ 3 was determined⁶, using recombinant DNA clones described previously^{7–9} (see Fig. 1). We have found several regions containing multiple repetitions of simple sequences (Fig. 2). The first of these, (CA)₂₄(TA)₁(CA)₈, referred to here as the (CA)₃₃ repeat, is located between the C_μ secreted (μ_s) and membrane (μ_m) regions beginning at base 1,097. Preceding this sequence is a region of about 1 kilobase (kb) that is rich in alternating pyrimidine-purine stretches (underlined in Fig. 2). These sequences along with the (CA)₃₃ repeat could possibly form Z-DNA¹⁰. A repeated hexamer, (GGGAGA)₁₂, starts at base 2,556, about 450 bases to the right of μ_m . Nine bases further 3' is (GA)₂₈. The second intron of C_δ contains two repeated sequences in immediate succession, (CT)₃₀ and (CA)₃₀. The latter may also have Z-DNA-forming potential. To determine whether copies of the repeated sequences occur elsewhere in the genome, hybridization experiments were carried out using fragments of DNA containing these repeats as probes of Southern transfers¹¹ of genomic DNA (Fig. 3C) and conversely using nick-translated genomic DNA hybridized to Southern transfers of digested plasmid subclones (Fig. 3A). As anticipated from similar studies on other eukaryotic genes^{12–16}, the simple repeats occur in multiple copies in the genome.

About midway between μ_m and C_δ 1 a 162-base sequence, unique-sequence inverted repeat (USIR), begins on the coding strand at position 3,519 and is inverted on the complementary strand starting at position 3,936. The two copies show 77% homology (see Fig. 2). In Southern hybridization experiments, this sequence appears to be unique to the μ - δ region (e in Fig. 3A, C).

We have searched for the pentamers TGGGG and GAGCT which are the main subunits of the S_μ region and other variants which are repeated in other S sites, such as GAGYT (Y = pyrimidine) and GGGYT³. We have also scanned for TGGG, TGAG^{3,4} and YAGGTG¹⁷ which have been identified near

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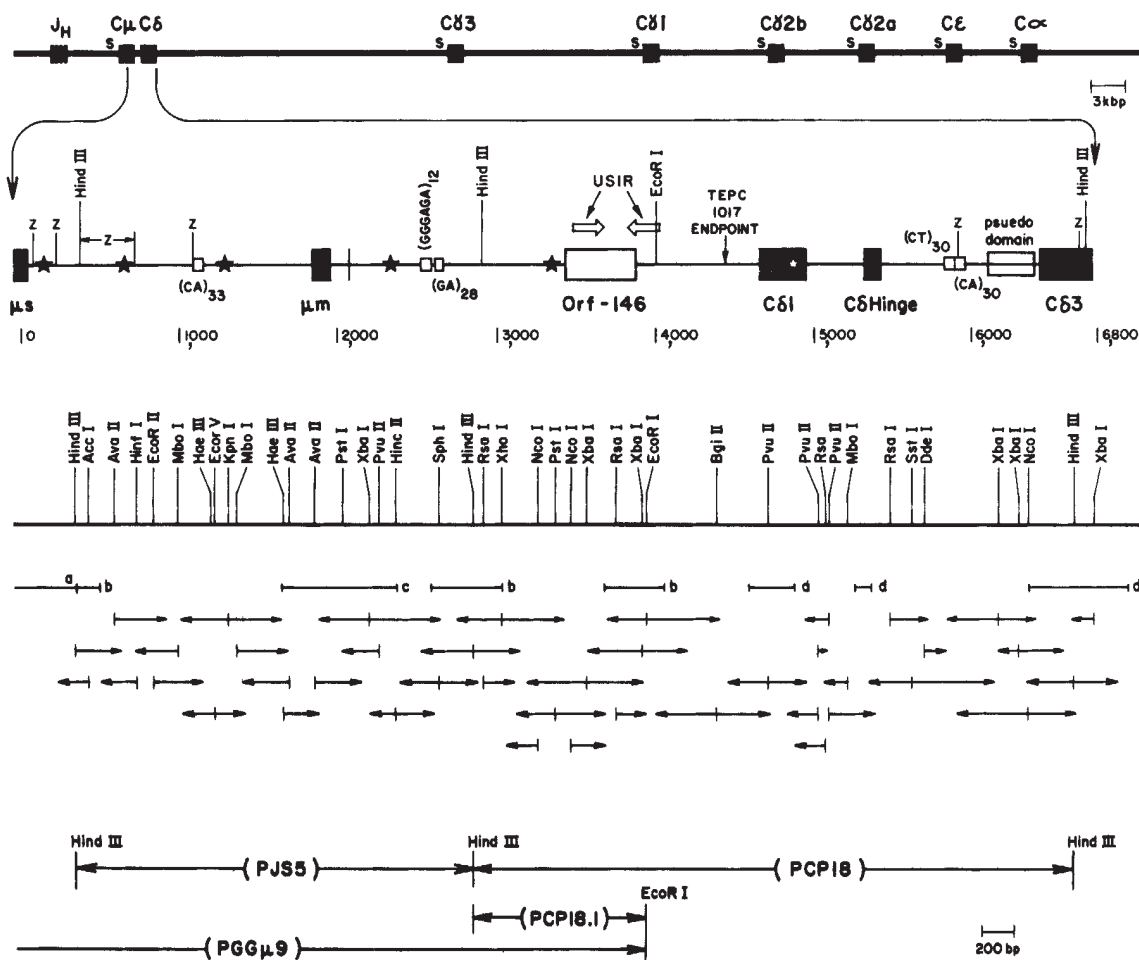


Fig. 1 Map of the μ - δ region and sequence strategy. The upper panel shows a scale map of the entire immunoglobulin H-chain locus. Coding sequences are shown as boxes and switch recombination regions are indicated by S. Previously published gene order and separation have been recently reviewed³⁷. The middle panel presents an enlargement of the μ - δ region (Fig. 2) and summarizes its unusual structural features. Tall filled boxes indicate established coding sequences (μ_s = secreted carboxyl terminal and μ_m = membrane carboxyl-terminal exons for C_μ). Tall open boxes represent the potential coding region, ORF 146 and the pseudodomain within the large intron of C_δ . Stars represent AATAAA polyadenylation-termination sequences²⁶ (potential End sites); 'Z' denotes alternating purine-pyrimidine stretches which might form Z-DNA. Short open boxes indicate simple repeated sequences whose compositions are given above or below. USIR, unique-sequence inverted repeat. The lower panel gives a detailed restriction map and the sequencing strategy used. Vertical lines indicate ³²P-labelled ends and arrows indicate the length and direction of sequences obtained. All restriction sites were crossed and 95% of the sequence was repeated on both strands. Regions sequenced by others are shown as lines without arrows. References are as follows: (a) ref. 38; (b) ref. 4; (c) ref. 39; (d) ref. 7. Plasmid subclones used in sequencing (pJS5, pCP18, pCP18.1 and pGGμ9) are indicated at the bottom.

the point of recombination in some myelomas. GAGCT occurs only once in 2,543 bases; in the same interval TGGGG occurs at only three well separated sites and YAGGTTG does not occur at all. Although S-site pentamers are nearly absent between μ and δ , the hexamer repeat (GGGAGA)₁₂ and the dimer repeat (GA)₂₈ are in this area just downstream of μ_{m2} (Figs 1, 2, 3B). Note that an identical counterpart of the dimer, (GA)₂₇, is located approximately 500 bp 5' to the $C_\delta 3$ gene (J. Wels, personal communication). A close variant of the hexamer repeat, (GGAGA)₂₄, is located within the S_α region about 2.1 kbp 5' to C_α (ref. 18). Recombination between the two dimer repeats or between the two hexamer repeats would in effect replace C_δ with $C_\gamma 3$ or C_α allowing for co-expression of μ and $\gamma 3$ or μ and α from a transcription unit which would be similar in size to that of μ - δ . However, there is no evidence for such a deletion.

The myeloma TEPC 1017 expresses only IgD¹⁹ and has been shown by analysis of Southern transfer hybridizations to have deleted the C_μ gene from all three rearranged copies of chromosome 12 (A.C.G., unpublished observations). By sequencing across the deletion junction in TEPC 1017, we have mapped the 3' end point between bases 4,394 and 4,395 of the germ-line sequence (Fig. 2). This is 255 bases 5' to $C_\delta 1$ in a region of

unique sequence (c in Fig. 3A, C). Thus, the deletion of C_μ in this plasmacytoma is not mediated by S-site sequences, at least on the 3' side. Analysis of the other side of the cloned TEPC 1017 rearrangement requires determination of sequences 5' of C_μ and will be presented elsewhere (Gilliam, A. C. *et al.*, submitted for publication).

Our scan for ORFs identified a region of 146 amino acids, ORF 146, which has many gene-like properties. It is located between C_μ and C_δ (bases 3,458–3,899 in Fig. 2) and is oriented in the same transcriptional direction as these genes. Part of its coding region extends into the unique-sequence inverted repeat. The codon usage profile (data not shown) is similar to that observed for other mammalian genes²⁰. It has the preferred consensus sequence²¹ spanning its AUG initiation codon. Chou-Fasman analysis²² of ORF 146 predicted β -sheets interspersed with β -turns as for immunoglobulin and related domains²³. A pair of cysteines 47 residues apart could provide an immunoglobulin-like disulphide loop.

Although ORF 146 has considerable homology and is probably evolutionarily related to immunoglobulins, it is extremely unlikely that it is incorporated into C_δ . The most direct experimental evidence comes from r-loop analysis²⁴ in which an ORF 146 exon is not visible. Alternatively, ORF 146 could be

Fig. 2 DNA sequence of the region from μ_s to the end of C_s3 . Amino acid sequences predicted for the μ_s , μ_m and C_s coding regions and for ORF 146 are shown directly above the first base of the corresponding codon in single-letter code: Phe, F; Leu, L; Ile, I; Met, M; Val, V; Ser, S; Pro, P; Thr, T; Ala, A; Tyr, Y; His, H; Gln, Q; Asn, N; Lys, K; Asp, D; Glu, E; Cys, C; Trp, W; Arg, R; Gly, G. Dashes between putative ORF 146 amino acids indicate a hydrophobic stretch of 27 residues at the carboxy terminus which could serve as a membrane-binding segment. Six potential End sites (aataaa)²⁶ are indicated in lower-case type, but only those at positions 163 and 2,348 are used *in vivo* to define the 3' end of μ_s and μ_m mRNAs (see Figs 1, 3 and refs 40, 41). The others may be involved in production of minor RNAs or, in the case of the one at position 4,895 (downward arrow), ORF 146. Nothing in the immediate vicinity of the two expressed End sites distinguishes them from the others. Translation terminators (TGA) for μ_s and μ_m are indicated in lower case and a putative TGA stop codon for ORF 146 is denoted by a dot. Regions of potential Z-DNA are underlined. Established RNA splice sites are indicated by an upward arrow below the junctional base. A search for splice sites using the 'perception' algorithm⁴² revealed the indicated splice sites plus an additional unused donor splice site at base 1,186 and an additional eight unused acceptor splice sites at bases 857, 1,733, 2,231, 2,862, 3,361 and 3,637 (noted by downward arrows for possible involvement with ORF 146 expression), 3,945 and 6,190. Comparisons (unpublished results) revealed no difference between functional and cryptic splice sites. None of the cryptic ones are identical to any of the 81 sites used for splicing other C_H genes, but several had very high statistical scores. The endpoint of the TEPC 1017 deletion between bases 4,394 and 4,395 is indicated by a upward arrow. Bases of the unique-sequence inverted repeat which could pair to form a stem are indicated by > or <. The sequences of μ_s (bases 1-393) and the C_s exons (4,653-4,934, 5,311-5,415 and 6,415-6,735) have been reported previously^{3,38} and with the sequence previously in exact position of the 5'-acceptor

expressed in three ways. (1) It could be autonomously transcribed using a T+A-rich region upstream of the putative cap site as a TATA box²⁵ and the AATAAA²⁶ (End site in Figs 1, 2) in C₆1 as a terminator (Fig. 2). (2) ORF 146 could be spliced to upstream exons at one of the two putative acceptor-RNA splice sites (downward arrows in Fig. 2), but this would eliminate

[illegible]

the amino-terminal half of the ORF. Moreover, if spliced to the J_H or C_μ exons the phase of translation of ORF 146 would be incorrect. (3) An ORF 146 message could be derived by cutting a precursor at End sites (positions 3,367 and 4,895 in Fig. 2). Such End-End fragments are expected if RNA polymerase continues down its DNA template beyond the End site.

Fig. 3 Characterization of the repeated sequences in the μ - δ region by hybridization. **A**, Hybridization of genomic DNA to Southern transfers of cloned fragments. Subcloned restriction fragments were isolated from genomic clones indicated by lines between the map in **b** and the bottom of the photographs. **a-d**, 1–3 μ g of each DNA was digested with various restriction enzymes identified above the photographs (H, *Hind*III; R, *Eco*RI; B, *Bam*HI), electrophoresed on 1.2% agarose, transferred to nitrocellulose and hybridized with *Hind*III-digested BALB/c mouse liver DNA, nick-translated⁴⁴ (1×10^8 c.p.m. μ g⁻¹) with [α -³²P]dCTP. Hybridization was carried out for 12–18 h at 65 °C in 3 $\times$ SSC, 0.1% SDS, 0.02% each of Ficoll, bovine serum albumin and polyvinyl pyrrolidone, with 100 μ g ml⁻¹ of tRNA as carrier. Filters were washed for 1 h in 3 $\times$ SSC, 0.1% SDS at room temperature, then for 1 h in 0.1 $\times$ SSC, 0.1% SDS at 42 °C, exposed to Kodak XAR film with intensifying screens at -70 °C. The autoradiograph for each experiment is shown beside the corresponding photograph of the agarose gel. Positive hybridization of probe DNA to a particular band indicates that it contains repetitive sequences. **a**, 0.7-kbp *Hind*III fragment containing a major portion of S_{μ} subcloned into pBR322. **b**, pJ55, a 2.7-kbp *Hind*III fragment containing the (CA)₃₃ repeat, μ_{m1} , μ_{m2} and the (GGGAGA)₁₂ repeat subcloned into pKB358 was cut with *Rsa*I. **c**, pCP18, a 3.9-kbp *Hind*III fragment containing the unique-sequence inverted repeat, the C_{δ} exons and the (CT)₃₀ and (CA)₃₀ repeats, subcloned into pBR322. **d**, 0.9-kbp *Bam*HI fragment containing the (GA)₁₆ repeat and the δ_{ϵ} exon⁴⁵ subcloned into pBR322. **B**, Map of the germ-line BALB/c mouse C_{μ} - C_{δ} gene complex. C_{μ} coding sequences are denoted by open boxes; C_{δ} coding sequences by filled boxes. Dashed lines indicate the length of S_{μ} in our clone⁹ of the region, which has a deletion relative to the germ-line length. ●, End sites. DNA fragments containing repeated sequences used as probes in **c** are indicated on the map. The approximate sizes of repeated sequences (~~~~) relative to probe sizes (-----) are shown. **C**, Hybridization of repeated sequences to Southern transfers of mouse germ-line DNA. Approximately 20 μ g of BALB/c mouse liver DNA were digested with various restriction enzymes indicated above each lane, electrophoresed, transferred to nitrocellulose and hybridized as described for **A** with ³²P-labelled fragments containing repeated DNA sequences. The probe fragments are as follows: **a**, (CA)₃₃ repeat, *Rsa*I 450 bp; **b**, (GGGAGA)₁₂ repeat, *Rsa*I 220 bp; **c**, unique-sequence inverted repeat, *Xho*I-*Bgl*II 1,360 bp; **d**, (CT)₃₀-(CA)₃₀ repeat, *Sst*I 620 bp. A blur of hybridization indicates that the probe contains repeated sequences found on many different sizes of restriction fragments in the germ-line DNA.

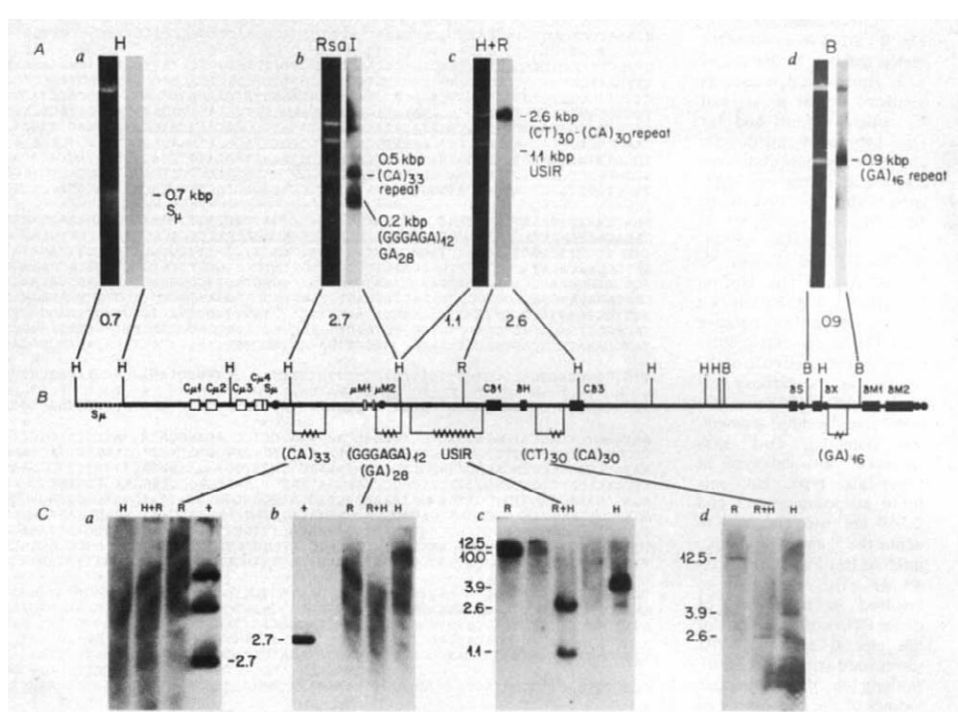
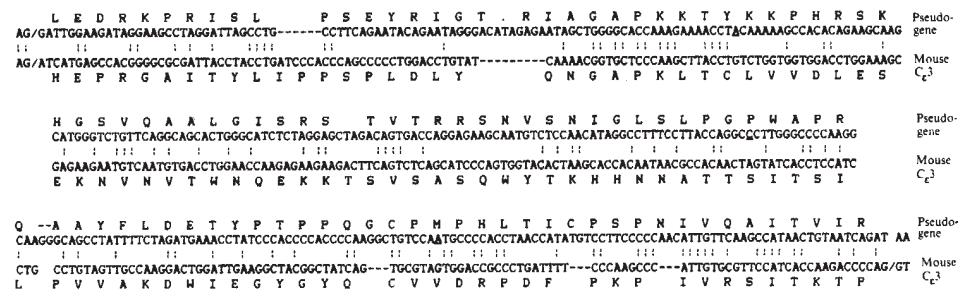


Fig. 4 Alignment of the pseudogene within the large C_{δ} intron with $C_{\delta}3$. The upper and bottom lines of each panel give the predicted amino acids (see Fig. 2 for abbreviations) for the mouse $C_{\delta}3$ domain²⁹ (second line) as compared with nucleotides 6,140–6,404 (third line) between $C_{\delta}H$ and $C_{\delta}3$ (Figs 1–3). Gaps (horizontal dashes) were inserted to maximize the number of base matches (vertical dashes). Underlined bases indicate where single-base changes in the pseudogene would yield an amino acid match for highly conserved domain residues. The alignment with $C_{\delta}3$ shows a similarity of 43.2% for DNA and 20.2% for protein (six gaps).



Similar processing of a μ transcript at a pair of End sites further upstream might also explain a recent report of a μ_m -containing RNA fragment in T cells²⁷.

The structure of mouse δ is unusual because it lacks a second constant-region domain⁸, which is present in human δ ²⁸ and all other mouse immunoglobulin heavy chains. Inspection of the sequence in Fig. 2 confirms that there is no ORF that could encode a functional domain between the hinge and $C_{\delta}3$. However, just to the right of the (CT)₃₀-(CA)₃₀ repeat we have found a region of homology that resembles $C_{\delta}3$ (ref. 29). The alignment shown in Fig. 4 gives a DNA homology of 43.2% and an amino acid homology of 20.2% (including six gaps). This homology to $C_{\delta}3$ is particularly interesting because human $C_{\delta}2$ is quite similar to human $C_{\delta}3$ at the protein level²⁸. Human $C_{\delta}2$, on the other hand, shares only a 12.3% homology with the amino translation of the mouse pseudodomain. The $C_{\delta}3$ pseudodomain might be an evolutionary relic of a formerly expressed $C_{\delta}2$ exon similar to the domain relic postulated for mouse $\gamma 2b$ (ref. 30). Since rat δ also lacks a $C_{\delta}2$ domain³¹, the loss of the functional $C_{\delta}2$ exon probably occurred before diver-

gence of the two species. It is interesting to note the proximity of this domain relic to the (CT)₃₀-(CA)₃₀ repeat which is related to simple sequence repeats shown to serve as 'hot spots' for recombination and gene conversion¹³. Inspection of the sequence immediately spanning this repeat revealed a perfect duplication (underlined) of nine bases: CCTAACTATT(CT)₃₀(CA)₃₀(GA)₄GCCTAACTA. Such terminal repeats are generated by insertion of mobile sequences, such as transposons, into staggered double-stranded breaks. Integration of the (CT)₃₀-(CA)₃₀ sequence into or near a formerly expressed $C_{\delta}2$ exon might also contribute to the loss of this domain in rodents.

The biological significance of several of the unusual sequence features found between C_{μ} and C_{δ} (Fig. 1) is not clear. At the level of chromatin structure, Z-DNA in the midst of B-DNA may enhance or depress transcription by relieving stress due to negative supercoiling^{10,32}. We have found potential Z-DNA within a relatively small region between C_{μ} and C_{δ} . As demethylation of the μ - δ region correlates with expression³³ and methylation enhances Z-DNA formation³⁴, it is possible that a flip

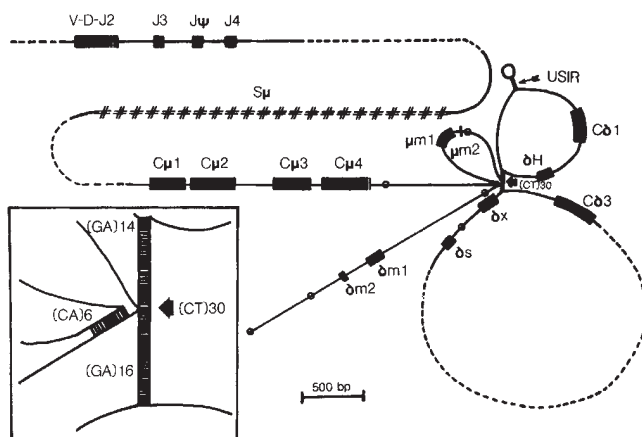


Fig. 5 Hypothetical folding of the μ - δ primary transcript. ■, Coding sequences^{8,38,39,45,46}; ○, end sites. Solid lines represent completed DNA sequence whereas incomplete sequence is indicated by broken lines. The S_{μ} region, which is frequently deleted in cloning, is shown here as full length. Complementary sequences of the unique-sequence inverted repeat (USIR) pair to form a 162-bp stable stem. The (GA)₂₈ simple sequence repeat (bases 2,636–2,691 in Fig. 2) can pair with the (CT)₂₈ simple sequence repeat (bases 5,851–5,910 in Fig. 2) to form a second stem. This double stem structure has been observed in heteroduplexes⁷. The (GA)₁₆, which has been reported near the δ_x exon⁴⁵, can also pair with the same (CT)₃₀ repeat, and the (GT)₆ repeat which occurs 20 bases to the right of (GA)₁₆ can pair with any part of the (CA)₃₃ repeat. Inset, expansion of the region of maximum pairing as indicated by the large arrow.

from Z- to B-DNA via methylation affects transcription past C_{μ} into the C_{δ} region. It is also tempting to speculate, based on studies in bacteria³⁵ and eukaryotes³⁶, that the large unique-sequence inverted repeat may serve to slow down or stop RNA polymerase at a stage in B-cell development when only μ mRNA is expressed.

Finally, a regulatory decision must be made in B cells in order to splice μ sequences from μ - δ -containing primary transcripts to produce δ mRNA. Our analysis of RNA splice sites in this region suggests that those which are used cannot be distinguished from those which are not used on the basis of sequence alone. Thus overall secondary structure of the RNA might provide the necessary component of specificity needed to determine the splice site. This could be accomplished if sequences between μ and δ and elsewhere in the longer μ - δ RNA precursor interact to fold the transcript into a configuration that excludes μ exons from the splicing apparatus. Figure 5 shows a structure which is predicted upon the pairing of the simple sequence repeats that occur between and downstream of μ and δ . Although some of this extensive secondary structure has been observed at the DNA level⁷, a complete prediction and verification of the structure and its effect on splicing and termination awaits the completion of the sequence of the entire transcribed region.

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Phorbol ester and diacylglycerol induce protein phosphorylation at tyrosine

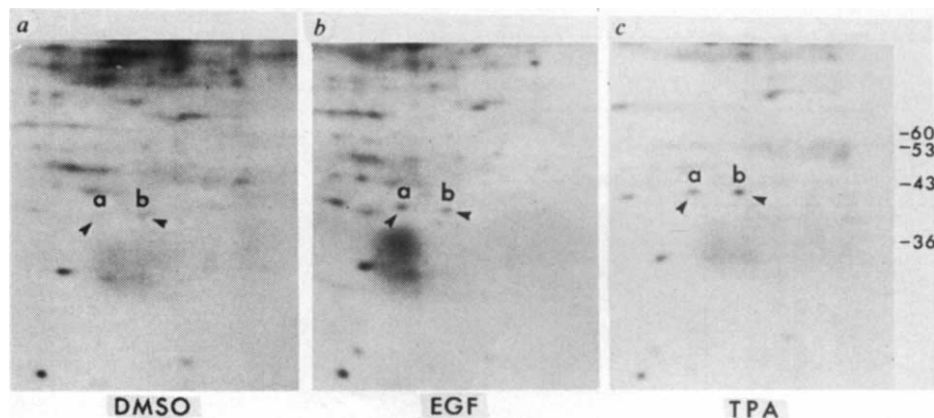
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The phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) is an efficient tumour promoter *in vivo*. *In vitro*, TPA activates the phospholipid- and Ca^{2+} -dependent protein kinase, kinase $C^{1,2}$. This activation is believed to reflect the structural similarity between TPA and diacylglycerol, the endogenous protein kinase C activator which is produced *in vivo* by hydrolysis of phosphatidylinositol (reviewed in ref. 3). Protein kinase C phosphorylates protein substrates at serine and threonine residues *in vitro*². The effects of TPA on cultured fibroblasts—including enhanced hexose uptake, disruption of actin stress fibres and growth stimulation—are very similar to those induced by certain retrovirus transforming proteins and by peptide growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and multiplication-stimulating activity (MSA)^{4–7}. These transforming proteins and mitogenic agents seem to act by inducing tyrosine-specific protein phosphorylation^{8–12}. Such observations suggested that some of the effects of TPA *in vivo* may be mediated by protein phosphorylation at tyrosine residues. A 42,000-molecular weight (42 K) polypeptide was previously shown to be phosphorylated at tyrosine in cells transformed by avian sarcoma viruses¹⁰ and in cells stimulated by EGF, PDGF or MSA (J. Cooper, personal communication and refs 11 and 12; this polypeptide was originally designated 43 K or spot n in ref. 10). We show here that this polypeptide also becomes phosphorylated at tyrosine in cells treated with TPA. Furthermore, exogenously added diacylglycerol likewise stimulates the phosphorylation of this protein at tyrosine.

To compare the effects of TPA and EGF on tyrosine-specific phosphorylation, chick embryo fibroblasts (CEF) were prelabelled for 6 h with ³²P-orthophosphate in serum- and phosphate-free medium, to equilibrate the radiolabel in the ATP pool¹³,

Fig. 1 Analysis of radiolabelled phosphoproteins in EGF- or TPA-treated CEF by two-dimensional PAGE. Secondary cultures of CEF were seeded at 10^6 cells per 35-mm dish in Dulbecco's modified Eagle medium supplemented with 1% calf serum and 1% heat-inactivated chick serum. On day 3 after plating, when the cultures had reached a density of $\sim 2.3 \times 10^6$, the cells were prelabelled for 6 h with 0.33 mCi ^{32}P -orthophosphate (ICN) in 1 ml of serum- and phosphate-free medium. These resting cultures were then treated with: a, DMSO (final concentration, 0.005%); b, EGF (Collaborative Research; final concentration 500 ng ml $^{-1}$); or c, TPA (Sigma; final concentration, 50 ng ml $^{-1}$); the reagents were added directly to the labelling medium. After 1 h, the cells were lysed as described previously^{15,25}. The samples were lyophilized, resuspended in pH 6–8 lysis buffer and analysed by two-dimensional PAGE as described previously^{15,25}. Samples ($\sim 100 \mu\text{g}$ protein) were separated in the first dimension by isoelectric focusing pH 6–8, and in the second dimension by electrophoresis on SDS-polyacrylamide gels containing 15% acrylamide and 0.09% methylene bis-acrylamide. The gels were fixed, stained with Coomassie blue and dried. The dried gels were then incubated in 1 M KOH at 55 °C for 2 h, neutralized in 10% acetic acid/10% isopropanol, and dried¹⁰. Gels were exposed to film for 48 h using a Lightning-plus (Du Pont) intensifying screen. A section of the autoradiogram (corresponding to the pH range 6.7–7.5) is shown above: the acidic end of the first dimension is to the left and the basic end to the right; molecular weight markers ($\times 10^{-3}$) are shown at the right of the figure. Arrowheads indicate the 42 K phosphoproteins, designated a and b in the figure.



and then treated with 50 ng ml $^{-1}$ TPA or 500 ng ml $^{-1}$ EGF. One hour later, radiolabelled phosphoproteins were analysed by two-dimensional polyacrylamide gel electrophoresis (PAGE) and autoradiography; to enrich for phosphoproteins containing phosphotyrosine, the gels were treated with alkali as described by Cooper and Hunter¹⁰. As shown in Fig. 1, two 42 K phosphoproteins are detected in TPA- and EGF-treated CEF; these are indicated as spots a and b in the figures (spot b is spot n of ref. 10). In these experiments dimethyl sulphoxide (DMSO) was used as the solvent for TPA. DMSO at high concentrations has been reported to induce tyrosine phosphorylation in membranes¹⁴. However, in CEF, DMSO alone at concentrations of 0.005% (Fig. 1a), 0.4% (Fig. 4a) or up to 5% (not shown) did not induce the phosphorylation of the 42 K polypeptides. Untreated cultures, or cultures treated with the biologically inactive phorbol ester 4- α -phorbol-12,13-didecanoate, showed only the same low level of phosphorylation of these proteins as that observed in DMSO-treated cells (not shown). The phosphorylation appears to occur *de novo*: analysis of TPA-treated cultures prelabelled with ^{35}S -methionine shows the appearance of spots in the positions of the 42 K phosphoproteins (not shown). We found no evidence for the phosphorylation of the 36,000-molecular weight polypeptide previously reported to be a major substrate for tyrosine phosphorylation in cells transformed by avian sarcoma viruses^{15,16}, nor for phosphorylation of any other of the substrates identified in retrovirus-transformed cells by alkali digestion of two-dimensional polyacrylamide gels¹⁰.

The relative mobilities of the 42 K phosphoproteins on two-dimensional gels suggested that they were the same as those which appear in avian sarcoma virus-transformed CEF (ref. 10, T.G., unpublished results, and J. Cooper, personal communication). Partial proteolytic digestion with *Staphylococcus aureus* protease V8 confirmed that this was the case (not shown).

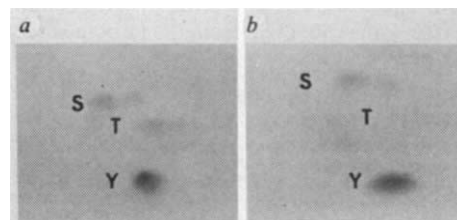
The relative mobilities of the 42 K phosphoproteins on two-dimensional gels suggested that they were the same as those which appear in avian sarcoma virus-transformed CEF (ref. 10, T.G., unpublished results, and J. Cooper, personal communication). Partial proteolytic digestion with *Staphylococcus aureus* protease V8 confirmed that this was the case (not shown).

Mapping of ^{32}P -orthophosphate- and ^{35}S -methionine-labelled phosphoproteins by partial proteolysis also indicated that the two 42 K polypeptides (spots a and b) are related to each other (not shown; J. Cooper, personal communication), suggesting that the more acidic spot probably represents a more highly phosphorylated species of the same polypeptide present in the less acidic spot.

To determine the phosphoamino acid content of these polypeptides, the 42 K phosphoproteins from TPA-treated cells were excised from the alkali-treated gels and subjected to partial acid hydrolysis. Both were found to contain phosphotyrosine as their major phosphoamino acid, plus minor amounts of phosphoserine and phosphothreonine (Fig. 2). It is possible that these phosphoproteins contain significant amounts of phosphoserine and phosphothreonine which are lost during the alkali hydrolysis; on non-alkali-treated gels the 42 K proteins appear as very minor species¹⁰ which are difficult to excise accurately, and hence it is not possible to determine the phosphoamino acid composition of the protein before alkali treatment. Laszlo *et al.*¹⁷ have shown that exposure of CEF to TPA for 18 h does not elevate the total level of phosphotyrosine in protein; this presumably reflects the fact that the 42 K polypeptides are minor phosphoproteins and that the phosphorylation is transient (see below). Our results do not exclude the possibility that there are other minor phosphoproteins containing phosphotyrosine which also appear following TPA treatment.

The kinetics of phosphorylation of the 42 K protein and the dependence of this phosphorylation on the concentration of TPA are shown in Fig. 3. The phosphoproteins appear rapidly after TPA treatment: phosphorylation is detectable after 5 min of treatment and maximal phosphorylation occurs by 15 min. Phosphorylation, although considerably diminished, is still detectable after 6 h (Fig. 3A–F). The two species seem to be phosphorylated in unison, that is, we did not detect a sequential

Fig. 2 Phosphoamino acid analysis of 42 K polypeptides from TPA-treated CEF. The phosphoproteins of TPA-stimulated CEF were radiolabelled and analysed as described for Fig. 1, except that the cells were labelled with 3 mCi ml $^{-1}$ of ^{32}P -orthophosphate. The autoradiograms were aligned with the dried alkali-treated gels to identify the position of the 42 K phosphoproteins. The spots (4 per sample) were excised from the gels: a, 42 K polypeptide designated a in Fig. 1; b, 42 K polypeptide designated b in Fig. 1. The proteins were eluted, precipitated with 20% trichloroacetic acid, washed with ethanol and hydrolysed in 6 M HCl at 110 °C for 80 min. The phosphoamino acids were separated by electrophoresis in two dimensions on cellulose thin layers (first dimension at pH 1.9, from right to left; second dimension at pH 3.5, bottom to top) as described previously^{8,10,15}. Cold markers were identified by ninhydrin staining: S, Phosphoserine; T, Phosphothreonine; Y, Phosphotyrosine. Autoradiography with sensitized film and an intensifying screen was for 8 days.



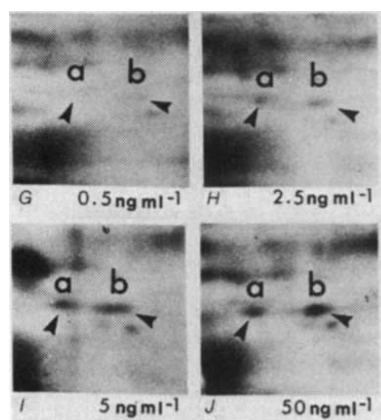
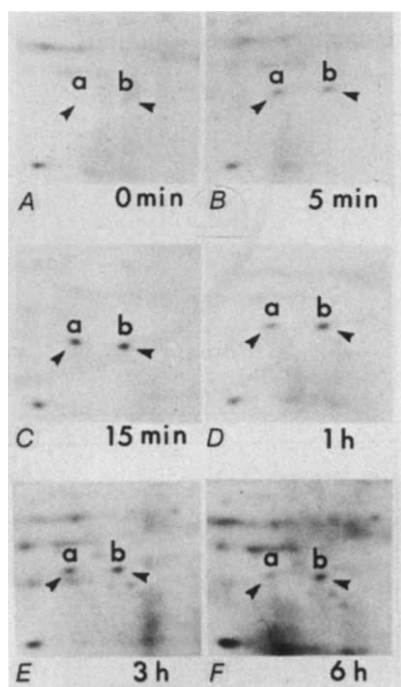


Fig. 3 Dependence of phosphorylation of the 42 K polypeptide on duration of exposure to TPA and on TPA concentration. Radiolabelled phosphoproteins were analysed as described for Fig. 1; only the pertinent portions of the autoradiograms are shown. **A-F**, Kinetics of phosphorylation of the 42 K polypeptide in CEF treated with 50 ng ml⁻¹ TPA. Prelabelled cells were lysed at the indicated times after addition of TPA. **G-J**, Dependence of phosphorylation of the 42 K polypeptide on TPA concentration. Prelabelled cells were treated with the indicated concentrations of TPA and lysed at 15 min after addition of TPA.

movement from a less to a more highly phosphorylated form as has been observed for the ribosomal protein S6 in similar conditions¹⁸. The phosphorylation of these polypeptides is half-maximal at 2.5 ng ml⁻¹ and near-maximal at 5 ng ml⁻¹ (Fig. 3G-J). The doses of TPA required to produce these effects are similar to those required to induce morphological changes, enhanced hexose uptake or stimulation of DNA synthesis⁴⁻⁷.

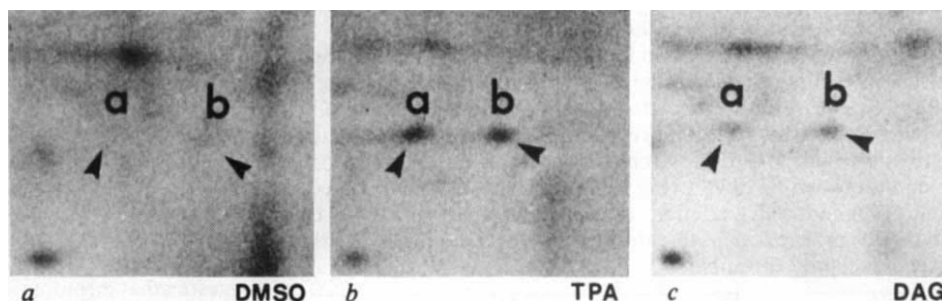
TPA induces a variety of rapid responses in platelets, and exogenously added diacylglycerol induces the same effects^{19,20}. To determine if diacylglycerol can also induce tyrosine phosphorylation in fibroblasts, we examined the effects of 200 µg ml⁻¹ 1-oleoyl-2-acetyl-glycerol on the phosphorylation of the 42 K protein. As shown in Fig. 4, diacylglycerol does indeed induce the appearance of the 42 K phosphoproteins. The relatively high concentration of diacylglycerol needed to produce this effect perhaps reflects the rapidity with which the exogenously added compound is metabolized; in contrast, TPA intercalates into the membrane bilayer and is metabolized only very slowly³.

The tyrosine phosphorylation of the 42 K protein represents a rapid response to the binding of TPA to its receptors. A variety of membrane receptors with intrinsic or associated tyrosine kinase activity have been identified, including those for EGF²¹, PDGF²² and insulin²³. However, the only known receptor for TPA is protein kinase C; as kinase C is a serine/threonine-specific kinase, it seems unlikely that the 42 K protein is directly phosphorylated by this enzyme. It is possible that TPA and diacylglycerol interact with kinases other than kinase C, and that a tyrosine kinase is directly activated by these agents. Alternatively, the activation of protein kinase C by TPA or diacylglycerol may result in the activation, directly or indirectly,

of one or more tyrosine-specific kinases. Another possibility is that TPA treatment inactivates a phosphoprotein phosphatase specific for the 42 K protein. As the identities of the kinases and phosphatases which act on the 42 K protein are unknown, it is not possible to distinguish between these possibilities. It is of interest that other mitogenic agents such as trypsin can also stimulate the tyrosine phosphorylation of the 42 K protein in CEF (B. Sefton, J. Cooper and T. Hunter, personal communication). It is not clear whether the phosphorylation of the 42 K protein is necessary for any of the phenotypic effects of virus transforming proteins or of non-viral mitogens. Nevertheless, this common response to such diverse agents suggests that tyrosine phosphorylation may represent a common element in the different pathways which lead to cell proliferation. In addition, because TPA induces a variety of responses in other cell types (for example, the differentiation of myeloid leukaemia cells²⁴ and exocytosis in platelets^{19,20}) and because protein kinase C is also activated by elevated intracellular Ca²⁺, it may be that tyrosine phosphorylation is also involved in mediating other types of cellular response.

While this manuscript was in preparation we learned that phosphorylation of the 42 K polypeptide in TPA-treated cells has also been observed by J. Cooper, B. Sefton and T. Hunter and by R. Bishop, R. Martinez, K. Nakamura and M. Weber; we thank Mike Weber, Bart Sefton, Jonathan Cooper and Tony Hunter for communicating their results before publication. We also thank Roger Tsien for stimulating discussions and the gift of synthetic diacylglycerol, Manfred Schliwa for advice and encouragement, Masae Namba for technical assistance, and Mina Bissell, Jeremy Thorner and Kathryn Radke for helpful criticism of the manuscript. This work was supported by NIH

Fig. 4 Stimulation of phosphorylation of the 42 K polypeptide by diacylglycerol. Prelabelled CEF were exposed for 15 min to **a**, 0.4% DMSO plus 4 µg ml⁻¹ bovine serum albumin; **b**, 50 ng ml⁻¹ TPA; or **c**, 200 µg ml⁻¹ 1-oleoyl-2-acetyl-glycerol (kindly provided by Roger Tsien) plus 0.4% DMSO and 4 µg ml⁻¹ bovine serum albumin. Phosphoproteins were analysed as described in Fig. 1 legend; only the relevant portions of the autoradiograms are shown.



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Phorbol ester-induced activation of human platelets is associated with protein kinase C phosphorylation of myosin light chains

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Phosphorylation of the 20,000 molecular weight (MW) light chain of platelet myosin is associated with the activation of platelets and subsequent release of platelet granules^{1–4}, and the protein kinase catalysing this phosphorylation has been identified as the Ca^{2+} /calmodulin-dependent enzyme, myosin light chain kinase^{5–9}. Tumour-promoting phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which activate Ca^{2+} -activated, phospholipid-dependent protein kinase (protein kinase C)¹⁰, can also cause platelet aggregation and phosphorylation of a 20,000-MW peptide in blood platelets^{11–13}. It was therefore of interest to ascertain whether the 20,000-MW peptide phosphorylated in platelets was the light chain of myosin and whether TPA-induced phosphorylation of the 20,000-MW peptide could be differentiated from thrombin-induced phosphorylation. We now report that TPA-induced activation of platelets is associated with the phosphorylation of the 20,000-MW light chain of myosin, that it appears to be mediated mainly through protein kinase C and that the site phosphorylated in the myosin light chain is distinct from that phosphorylated by myosin light chain kinase.

TPA, an activator of protein kinase C, triggers platelet aggregation as well as the secretion of [¹⁴C]-serotonin from platelets^{11–13}. The response to activation by 1 μM TPA at 37 °C, with constant stirring, is more gradual than the response to activation by 0.1 U ml^{-1} thrombin and maximal aggregation occurs between 8–10 min (Fig. 1). Carroll *et al.*¹³, also noted that TPA-induced secretion of serotonin from platelets is markedly reduced in both rate and extent as compared with that induced by thrombin.

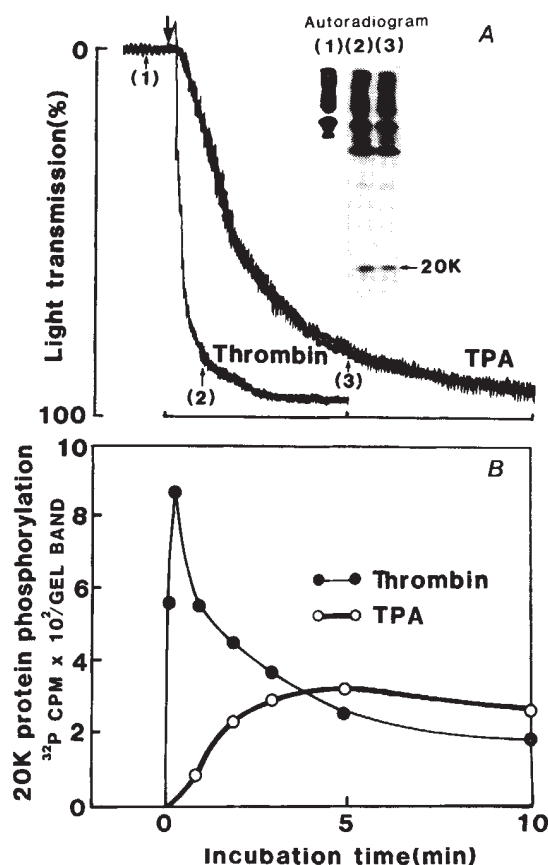


Fig. 1 Time-courses for aggregation (A), and phosphorylation of the 20,000-MW peptide (B), in platelets stimulated by 1 μM TPA or 0.1 U ml^{-1} thrombin.

Methods: The washed platelets (2×10^{10} cells) were incubated at 37 °C with [³²P]PO₄³⁻ (0.4 mCi ml⁻¹) in 10 ml of buffer A (0.15 M NaCl, 15 mM Tris-HCl (pH 7.4) and 5.5 mM glucose) as described by Lyons *et al.*¹. After 30 min of incubation, the suspension was sedimented (1,400g for 5 min). The supernatant solution containing unincorporated radioactivity was discarded. The platelet pellet was resuspended in 40 ml of buffer A (5×10^8 cells ml⁻¹). The platelets were preincubated in the aggregometer (Rikadenki) for 3 min before stimulation with TPA or thrombin. Platelet aggregation was studied by the turbidimetric method of Born¹⁶ using a Rikadenki 4 channel Aggregometer (RAM41). The standard platelet reaction mixture consisted of 0.5 ml of radioactive platelets (5×10^8 cells ml⁻¹), and 1 μM TPA or 0.1 U ml^{-1} thrombin (as indicated in the figure). To assay for phosphorylation of the 20,000-MW peptide the incubation was terminated by the addition of a half volume of a stock solution which contained 9% SDS, 6% 2-mercaptoethanol, 15% glycerol, 0.19 M Tris-HCl (pH 6.7). The sample was boiled in a waterbath for 1 min and subjected to SDS-polyacrylamide slab gel electrophoresis under the conditions described by Laemmli¹⁷. The separating and stacking gels contained 15 and 5% acrylamide, respectively. The gel was stained with Coomassie blue and after destaining, was exposed to Kodak X-Omat AR X-ray film. Based on the autoradiograph, the gel was cut and counted for ³²P in a Beckman liquid scintillation spectrometer. 20 K, 20,000-MW peptide.

Previous work has shown that thrombin- and TPA-induced activation of platelets is accompanied by the time-dependent incorporation of ³²P into peptides of MW 40,000 and 20,000. In the case of thrombin, the smaller peptide has been identified as the regulatory light chain of myosin^{5,8,9}, but the identity and function of the 40,000-MW protein are still uncertain. Figure 1A shows that either thrombin or TPA treatment of platelets results in the phosphorylation of a 20,000-MW peptide. Figure 1B shows that maximal phosphorylation of a 20,000-MW peptide occurred at 0.5 min with thrombin and 5 min with TPA. This marked difference in the time course of phosphorylation suggested that thrombin and TPA might mediate the phosphorylation of this peptide through different kinases. It was therefore

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Fig. 2 Two-dimensional chromatography of tryptic peptides of the ^{32}P -labelled 20,000-MW peptide. **A**, Tryptic phosphopeptides following incubation with TPA; **B**, tryptic phosphopeptide following incubation with thrombin; X indicates the origin.

Methods: Platelets, prelabelled with ^{32}P were activated by either TPA ($1\ \mu\text{M}$) or thrombin ($0.1\ \text{U ml}^{-1}$) at 37°C for 5 min or 0.5 min, respectively. Assay conditions were as in Fig. 1. The radioactive 20,000-MW peptide was isolated by SDS-polyacrylamide gel electrophoresis, eluted from the gel, and digested with trypsin. The radioactive tryptic phosphopeptides were then subjected to peptide mapping using silicagel thin layer plates, according to the procedures described by Yamaguchi *et al.*¹⁸. The first dimension was electrophoresis with acetic acid/formic acid/ H_2O (15:5:80) at 1,000 V. The second dimension was chromatography in *n*-butyl alcohol/pyridine/acetic acid/ H_2O (32.5:25:5:20).

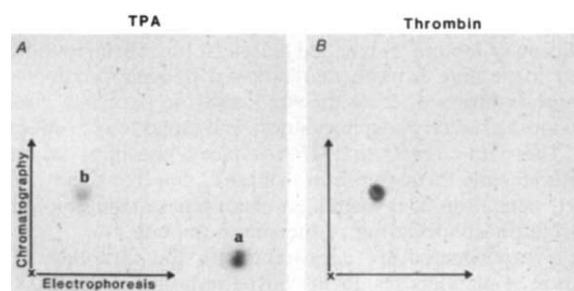
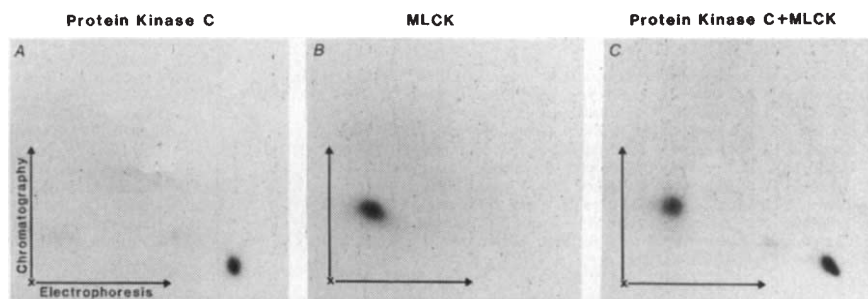


Fig. 3 Identification of the tryptic phosphopeptides following phosphorylation of platelet myosin with protein kinase C or myosin light chain kinase. Platelet myosin was prepared from fresh human platelets by the method described previously¹⁹. **A**, Tryptic phosphopeptide following incubation with protein kinase C; **B**, tryptic phosphopeptide following incubation with myosin light chain kinase; **C**, equal mixture of **A** and **B**.

Methods: Platelet myosin light chain kinase was purified to apparent homogeneity, as described by Hathaway *et al.*²⁰. Protein kinase C was partially purified from rat brains by the procedure of Schatzman *et al.*²¹. Platelet myosin ($1.3\ \text{mg ml}^{-1}$) was phosphorylated in $200\ \mu\text{l}$ of a reaction mixture containing 20 mM Tris-HCl (pH 7.5), 10 mM MgCl_2 , 0.3 mM CaCl_2 , 50 mM NaCl, 0.5 mM dithiothreitol, 1 mM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ ($0.3\ \text{Ci mmol}^{-1}$), 2 μM calmodulin or 50 $\mu\text{g ml}^{-1}$ phosphatidylserine plus 0.8 $\mu\text{g ml}^{-1}$ 1,3-diolein, and either 4 $\mu\text{g ml}^{-1}$ platelet myosin light chain kinase or 40 $\mu\text{g ml}^{-1}$ protein kinase C at 30°C for 30 min. The reactions were initiated by the addition of ATP and terminated by the addition of 40 μl of a 2% SDS solution containing 10 mM dithiothreitol and 20% glycerol. The phosphorylated light chain of platelet myosin was isolated following SDS-polyacrylamide gel electrophoresis and digested with trypsin. Two-dimensional peptide mappings were performed with silicagel thin layer plate, according to the procedures described by Yamaguchi *et al.*¹⁸.



of interest to compare the sites phosphorylated in the 20,000-MW peptide, following maximal stimulation by thrombin and TPA.

^{32}P -labelled platelets were activated by either TPA or thrombin, and the 20,000-MW peptide was eluted following SDS-polyacrylamide gel electrophoresis. This peptide was then digested with trypsin and the resulting radioactive peptides were analysed by two-dimensional mapping. Figure 2 shows that there is a marked difference in the map obtained following TPA and thrombin-induced activation of platelets. In the former case, two radioactive peptides can be identified (Fig. 2A, a and b), with the majority of the counts present in peptide a. A single phosphopeptide was found following tryptic hydrolysis of the 20,000-MW peptide phosphorylated following thrombin treatment (Fig. 2B). Although this tryptic phosphopeptide appears to be similar to the minor phosphopeptide shown in Fig. 2A, it is clear that TPA-induced phosphorylation occurs mainly at a different site in the 20,000-MW peptide, than does thrombin-induced phosphorylation.

It was important to supplement these experiments, carried out with intact cells, with *in vitro* experiments using purified proteins. This would also help to provide evidence that the 20,000-MW peptide phosphorylated in Fig. 1 was in fact the light chain of myosin. Previous work¹⁴ had demonstrated that the isolated 20,000-MW light chain of chicken gizzard myosin, as well as intact gizzard myosin could serve as a substrate for protein kinase C. Kikkawa *et al.*¹⁵ had demonstrated that platelets contain a relatively large amount of protein kinase C and it appeared reasonable to assume that TPA was activating this kinase¹⁰.

We therefore conducted a series of experiments using purified human platelet myosin, in order to compare the sites phosphorylated by myosin light chain kinase and protein kinase C. When platelet myosin was incubated with either the Ca^{2+} /calmodulin-dependent enzyme, myosin light chain kinase or the Ca^{2+} /phospholipid-dependent enzyme, protein kinase C and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, ^{32}P was only incorporated into the 20,000-MW light chain of myosin (data not shown). Two-dimensional peptide mapping of

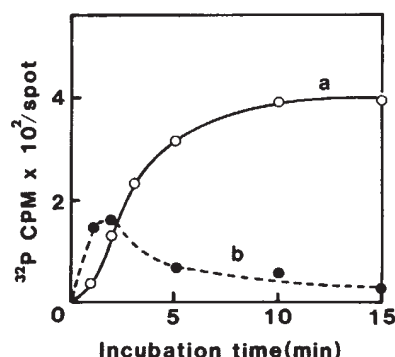


Fig. 4 Time-courses of TPA-induced phosphorylation of phosphopeptides b and a. ^{32}P -labelled platelets were activated at 37°C by $1\ \mu\text{M}$ TPA for various times as indicated. The assay conditions were as in Fig. 1. Two-dimensional mapping was performed as shown in Fig. 2. After two-dimensional mapping, the silicagel thin layer plate was exposed to an X-ray film to prepare the autoradiograph. The phosphopeptides identified in this manner were scraped from the thin layer plate and counted for ^{32}P in a Beckman liquid scintillation spectrometer.

the phosphorylated 20,000-MW light chain of myosin, following tryptic digestion, revealed that protein kinase C phosphorylated a different site on the 20,000-MW myosin light chain from myosin light chain kinase (Fig. 3). Moreover, the site phosphorylated by protein kinase C was the same as the major site phosphorylated in the 20,000-MW peptide following TPA-induced phosphorylation of intact platelets (compare Figs 3A and 2A). However, the minor phosphopeptide seen following tryptic digestion of TPA-stimulated cells (Fig. 2A, peptide b) appears to be from the same site that is phosphorylated by myosin light chain kinase (Fig. 3B). As expected, the site phosphorylated by purified myosin light chain kinase was the same as that phosphorylated in the myosin light chain following activation of platelets by thrombin (compare Figs 3B and 2B).

Figure 4 shows a time course of phosphorylation of the site located in peptide b as well as in peptide a, following TPA stimulation of intact ^{32}P -labelled platelets. Interestingly the site located in peptide b underwent a rapid phosphorylation and dephosphorylation whereas the site located in peptide a underwent a more gradual phosphorylation, and remained phosphorylated. The data suggest that TPA-induced phosphorylation is mediated mainly through protein kinase C, but that there is also an early activation of myosin light chain kinase resulting in the reversible phosphorylation of the site in peptide b.

These experiments are consistent with the idea that TPA activation of platelets results in phosphorylation of the 20,000-MW light chain of myosin, in addition to the phosphorylation of the 40,000-MW protein¹⁰. Phosphorylation of myosin light chain, which follows activation of the Ca^{2+} /phospholipid-

dependent kinase C, occurs mainly at a different site from that catalysed by myosin light chain kinase. The data reinforce the importance of identifying the site(s) phosphorylated on a given peptide following kinase activation. Although the physiological function of TPA-activated phosphorylation of the 20,000-MW light chain of platelet myosin remains to be elucidated, it seems reasonable to suggest that this phosphorylation, as well as that of the 40,000-MW protein, have a role in regulating platelet function.

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Regulation of *myc* gene expression in HL-60 leukaemia cells by a vitamin D metabolite

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HL-60, a cell line established from a patient with promyelocytic leukaemia, responds to a variety of inducing agents by ceasing division and acquiring some of the characteristics of either granulocytes or monocytes¹⁻⁴. Among the agents so far tested, only a comparative few occur naturally in vertebrates and would appear to have significant clinical potential in the treatment of leukaemic patients. One of the most promising of these is the dihydroxymetabolite of vitamin D₃, 1,25(OH)₂D₃. This compound circulates in normal man and has a major role in calcium homeostasis⁵. Moreover, it has recently been reported that 1,25(OH)₂D₃ increases the survival time of mice injected with myeloid leukaemia cells⁶. We⁷ and McCarthy *et al.*⁸ have previously shown that HL-60 cells respond to near physiological levels of 1,25(OH)₂D₃ by rapidly acquiring a number of monocyte-like features. Here we document that these phenotypic changes are preceded by a marked decrement in the expression of the *c-myc* oncogene. In fact, the diminution in the level of *c-myc* mRNA parallels the dose dependency and metabolite specificity shown by the various other indicators of phenotypic change. In addition, we demonstrate that removal of vitamin D₃ after the onset of maturational change, results in the reappearance of elevated *myc* mRNA levels. We believe this to be the first demonstration of a sequential relationship between the application of an exogenous inducing agent, a reduction in *myc* mRNA levels and the development of characteristics associated with normal cell maturation.

HL-60 is one of a comparatively small number of human leukaemic cell lines that has proven suitable for studying the chemically induced differentiation of myeloid cells *in vitro*. HL-60 is developmentally bipotential, giving rise to nonproliferative cells with some granulocytic characteristics in response to one set of inductive agents (for example dimethyl sulphoxide (DMSO), retinoic acid) and to cells with monocytic characteristics in response to another set (the phorbol esters)¹⁻⁴. Suda and his collaborators have shown that HL-60 cells contain cytosolic receptors for 1,25(OH)₂D₃ (ref. 9), and have reported that they differentiate into more mature cells of the myeloid series when exposed to this vitamin/hormone. Subsequently, we⁷ and McCarthy *et al.*⁸ confirmed the sensitivity of HL-60 to 1,25(OH)₂D₃ but found that the induced cells exhibited features of monocytes, not granulocytes. We identify induced HL-60 cells as granulocytic if they exhibit nuclear constriction and pycnosis, and show increases in chloracetate esterase and peroxidase histochemical staining. On the other hand, we regard induced cells as monocytic if they become positive for nonspecific esterase, synthesize and secrete lysozyme, lose peroxidase and chloracetate esterase activity, and acquire several cell-surface antigens that are extensively represented only in normal human monocytes.

The HL-60 genome contains several different oncogenes but only one, *c-myc*, is significantly amplified (≈ 16)^{10,11} and transcribed at high levels ($\approx \times 20$) (U. Rovigatti and S.M.A., unpublished). Westin *et al.*¹² recently reported that *myc* mRNA is no longer present in HL-60 cells rendered granulocytic by exposure to DMSO or retinoic acid. We wondered, therefore, whether a similar reduction in *myc* transcript level would be observed with vitamin D₃-induced monocytic differentiation and, more importantly, whether the diminution in *myc* mRNA levels would be an early event in the maturation sequence. The latter is clearly implied in one widely discussed hypothesis of carcinogenesis which states that cell transformation and malignancy may result from the elevated expression of an oncogenic locus¹³.

HL-60 cells were exposed to each of several different metabolites of vitamin D₃ or the ethanol carrier, and collected, after various time intervals, for analysis of induced maturational change and determination of *myc* mRNA levels. Maturational change was established by measuring lysozyme synthesis and secretion, α -naphthylacetate esterase activity and the appearance of two monocyte-associated cell-surface antigens, 63D3 (ref. 14) and Mac-120 (ref. 15). Levels of *myc* mRNA were

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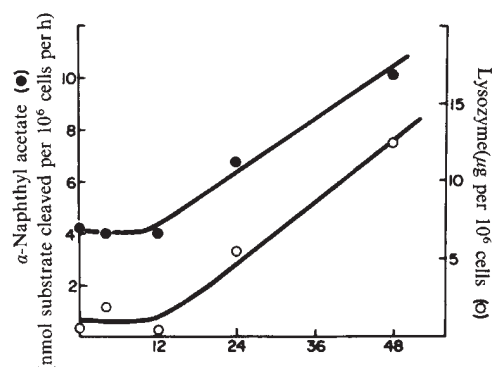


Fig. 1 The time course of appearance of nonspecific esterase activity and lysozyme in HL-60 cells treated with 5×10^{-8} M $1,25(\text{OH})_2\text{D}_3$. Note that both enzymes are unchanged during the first 12 h of incubation but increase significantly thereafter. Lysozyme activity is measured in aliquots of culture medium using *Micrococcus lysodeikticus* as a substrate and egg white lysozyme as a standard²⁰. Nonspecific esterase is determined from cell lysates using α -naphthyl acetate as a substrate and Fast blue RR as the chromogen. Each point represents the mean of triplicate cultures.

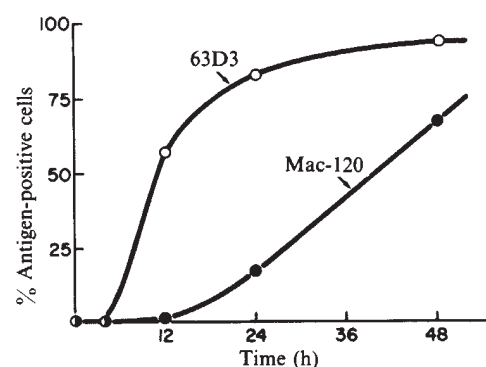


Fig. 2 The chronology of appearance of two cell-surface antigens in HL-60 cells treated with 5×10^{-8} M $1,25(\text{OH})_2\text{D}_3$. Note that the antigen Mac-120¹⁵ appears with a time course essentially identical to those of lysozyme and nonspecific esterase, but that 63D3¹⁴ develops much more rapidly and is, in fact, visible on more than 50% of the cells within 12 h of vitamin treatment. Antigens are detected by exposing the cells to primary antibody for 30 min at 4 °C, rinsing and then adding fluorescein isothiocyanate-labelled F(ab') rabbit anti-mouse immunoglobulin for another 30 min. Subsequently, the cells are rinsed again and then fixed and counterstained with 0.005% Evan's blue in 2% paraformaldehyde. Reactive cells are scored with a Zeiss epifluorescence microscope.

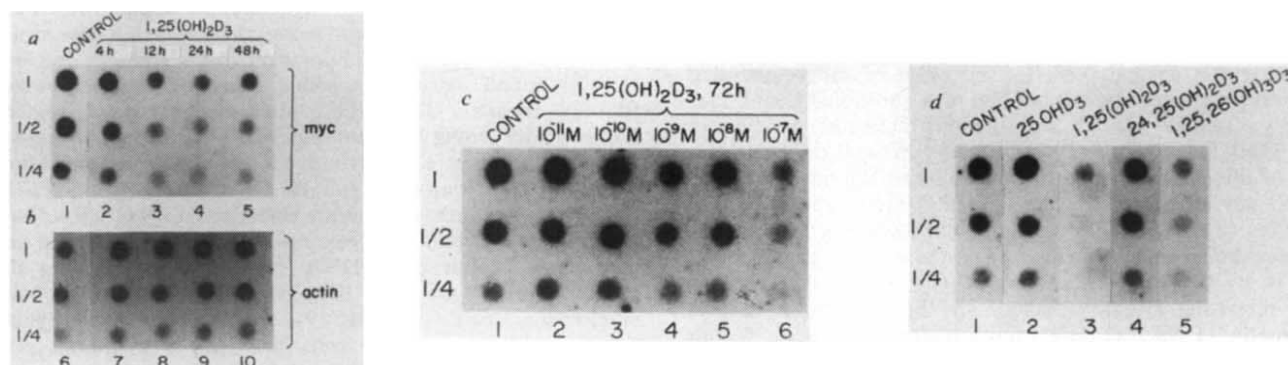


Fig. 3 RNA 'dot blots' from HL-60 cells following exposure to $1,25(\text{OH})_2\text{D}_3$. In this type of experiment, cells are collected after 4–72 h incubation with the vitamin D_3 metabolite, total RNA is extracted and then aliquoted onto nitrocellulose filters with and without further dilution ($\frac{1}{2}$; $\frac{1}{4}$). After baking, the filters are hybridized overnight at 42 °C with nick-translated DNA probes (4×10^6 c.p.m. ml^{-1}), and the unbound fraction is removed by extensive washing. The filters are then autoradiographed for various periods of time at –70 °C. The *myc* probe is a 1.7 kilobase *Cla*I–*Eco*RI fragment covering most of the 3' exon of the normal human *myc* gene¹⁶. Actin mRNA is probed with a 1.3-kilobase mouse α -actin clone²¹. Note that there is a significant reduction in *myc* level within 4 h of exposure to $1,25(\text{OH})_2\text{D}_3$ (a) whereas the level of actin mRNA does not change (b). RNA dot blots showing that the reduction in *myc* mRNA levels is specific for metabolites hydroxylated at the carbon 1, 24 and/or 25 positions (c; $25\text{OH}\text{D}_3$ and $24, 25(\text{OH})_2\text{D}_3$ at 10^{-6} M; $1,25(\text{OH})_2\text{D}_3$ and $1,24,25(\text{OH})_3\text{D}_3$ at 10^{-8} M; 72 h incubation. $1,25(\text{OH})_2\text{D}_3$ is effective in suppressing *myc* gene expression between 10^{-9} and 10^{-7} M (d), values that coincide with the minimal doses necessary to affect other phenotypic changes in HL-60 significantly⁷.

determined by 'dot blot' analysis of guanidium thiocyanate/ CoCl_2 purified RNA using a ^{32}P -labelled human c-*myc* DNA subclone to identify complementary nucleotide sequences¹⁶.

HL-60 cells respond to $1,25(\text{OH})_2\text{D}_3$ by quickly developing the four monocytic characteristics described above. Significant increases in secreted lysozyme (Fig. 1), cell-associated esterase activity (Fig. 1) and Mac-120-positive cells (Fig. 2) are detectable between 12 and 24 h of incubation and follow essentially identical kinetics. The 63D3 antigen, on the other hand, appears earlier (after 4–12 h of culture) and involves, initially, a much greater percentage of the cells than its Mac-120 counterpart (Fig. 2).

myc mRNA levels show an even more dramatic and rapid change in response to $1,25(\text{OH})_2\text{D}_3$ than do the monocytic markers. Assuming a steady rate of mRNA degradation, c-*myc* transcription is reduced to ~50% of initial values within 4 h and to ~20–30% by 12–24 h (Fig. 3a). This response is both metabolite specific (Fig. 3b) and concentration dependent (Fig. 3c) and is evidently not the result of a generalized shift in mRNA synthesis or degradation. Specifically, replicate RNA

dot blots probed for actin instead of *myc* sequences show no alteration in message level subsequent to induction (Fig. 3d).

The effect of active vitamin D_3 metabolites on HL-60 is ultimately irreversible, yielding a nonproliferative population after several days' exposure to the vitamin/hormone⁷. On the other hand, removal of $1,25(\text{OH})_2\text{D}_3$ after brief periods of incubation results in a mixed pattern of phenotypic change with the persistence of some of the characteristics of maturing cells (for example, 63D3 and altered G_0 – $\text{G}_1/\text{S}/\text{G}_2$ ratios; unpublished observations) but not of others. For example, *myc* mRNA content is the same in 48-h post-treatment HL-60 as in controls (Fig. 4).

Enhanced expression of the *myc* locus and oncogenesis have been noted in a variety of tumours, especially Burkitt's lymphomas and plasmacytomas¹⁷. However, for most of these cases, as with HL-60, it remains to be established whether elevated *myc* expression is the primary oncogenic event. (In fact, it has very recently been argued that there is no causal relationship between *myc* activity and Burkitt's lymphoma¹⁸.) As noted previously, Westin *et al.*¹² have shown that DMSO- or retinoic acid-induced, granulocytic HL-60 cells lack *myc* mRNA, and

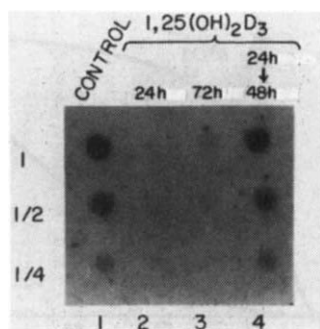


Fig. 4 RNA dot blots showing the reappearance of *myc* mRNA in treated HL-60 cells following the removal of $1,25(\text{OH})_2\text{D}_3$. At both 24 and 72 h of treatment, *myc* mRNA is virtually undetectable whereas removing the hormone at 24 h and subsequent culture for 48 h in control medium leads to reappearance of *myc* mRNA.

Astrin and Rovigatti¹⁹ have reported a diminution in *myc* transcription in lymphocytes isolated from a patient with B-cell lymphoma subsequent to clinical remission. Both findings and the present observations are clearly consistent with but do not prove a cause and effect relationship between *myc* expression and malignant behaviour.

We have shown here that $1,25(\text{OH})_2\text{D}_3$ reduces *myc* mRNA levels in HL-60 within 4 h of exposure to the vitamin/hormone, and that this change precedes the onset of other measured phenotypic changes by a minimum of 8 additional hours. Previously, Tanaka *et al.*⁹ had demonstrated the binding of $1,25(\text{OH})_2\text{D}_3$ to HL-60 cytosolic receptors and the translocation of this vitamin-receptor complex into the nucleus within 30–60 min of treatment. Thus, $1,25(\text{OH})_2\text{D}_3$ appears to be affecting cell maturation and gene expression in HL-60 in a manner similar to that of vitamin D_3 in other biological systems, that is, by first forming a complex with a cytosolic receptor, then migrating into the nucleus and finally interacting with chromatin (DNA) to alter transcriptional activity⁵. If this mechanism is correct for HL-60, the key question is whether the altered transcription is the consequence of hormone interaction solely with the *c-myc* promoter (as the signal event in the induction process) or a spectrum of promoters, each associated with a gene or gene cluster involved in determining cell phenotype. We are currently assessing these alternatives.

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Association of amplified oncogene *c-myc* with an abnormally banded chromosome 8 in a human leukaemia cell line

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Several unusual chromosome structures have been described in drug-resistant cell lines and in certain tumours. These structures include elongated homogeneously staining regions (HSRs), small extrachromosomal paired chromatin bodies (double minutes, DMs) and abnormally banded regions (ABRs) with strong but anomalous band patterns^{1–3}. There is evidence that these are alternative forms of gene amplification, with HSRs breaking down to form DMs, and DMs integrating into the chromosome to generate HSRs and ABRs^{5–7}. Recently, it was demonstrated that, compared with several normal and leukaemia human cells, DNA sequences representing the human homologue of the *onc* gene of the avian myelocytomatosis virus (MC29), the so-called *c-myc* gene, were amplified in HL-60 cells^{11,12}. This is a human promyelocytic leukaemia cell line established in the laboratory of one of us (R.C.G.) at the National Cancer Institute (Bethesda, Maryland) in 1977, and widely used for studies on myeloid and monocytic differentiation^{8–10}. Amplification of the gene was present in primary leukaemic cells of the patient¹², and DMs were noted in some of these cells as well as in early passages of the HL-60 line¹³. No structure resembling HSRs or ABRs were noted in karyotypic studies at this early stage¹³ and there were no alterations involving the long arm of chromosome 8 (8q), to which the *c-myc* gene has recently been mapped^{14–16}. We have now re-examined the karyotype of the HL-60 line, using cells frozen at various times during its continuous passage at the Wistar Institute (Philadelphia, Pennsylvania) to look for chromosomal abnormalities that might be associated with the amplification of *c-myc*. We find that, beginning in 1979, HL-60 cells at the Wistar Institute no longer had DMs, but did show an abnormal 8q⁺ chromosome, replacing a normal chromosome 8, and representing an ABR reflecting the site of *myc* gene amplification.

Our relevant chromosome findings and associated *c-myc* data are summarized in Table 1. The three samples of the HL-60 cell line that were frozen in 1978 at the National Cancer Institute (78B) or at the Wistar Institute (78A, 78C), and thawed shortly before our study, had an identical karyotype (Fig. 1). There were 44 or 45 chromosomes (half the cells were trisomic for chromosome 18), with loss of chromosomes X, 5, 9, 10 and 17, and the presence of three markers whose components we have tentatively identified (Fig. 1). Approximately 10% of the cells were tetraploid, and nearly all metaphases contained numerous DMs. In all three specimens, both chromosomes 8 appeared normal, and there was no evidence of involvement of segments from chromosome 8 in any of the translocations producing the marker chromosomes. Within the technical limitations of our

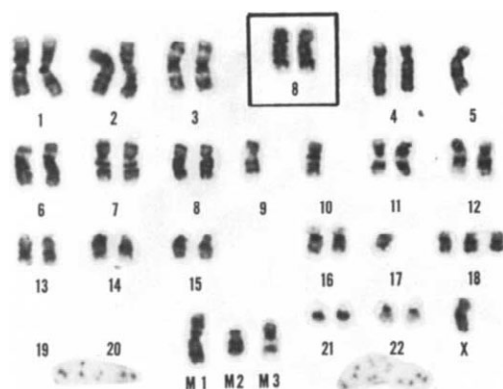


Fig. 1 Representative G-banded karyotype from cell line HL-60-78C, considered to be: 45, X, -X, -5, -9, -10, -17, +18, +mar1, +mar2, +mar3. A chromosome 14 may be abnormal. The three markers were noted previously by Gallagher *et al.*¹³; markers 1 and 2 are now tentatively identified as t(10q; ?13q) and 9p⁻, respectively; the two arms of marker 3 resemble 5p and 6p, suggesting derivation from one or both of these chromosomes. Nearly all metaphases contained many DMs (shown at bottom). Two normal chromosomes 8 from another cell are also illustrated (inset, top).

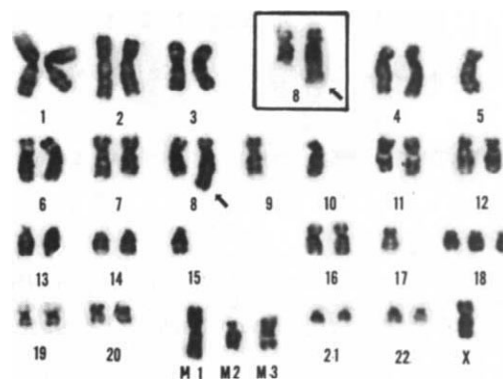


Fig. 2 Representative G-banded karyotype from cell line HL-60-79B. It is identical with the HL-60-78 lines (Fig. 1) except for an 8q⁺ (arrow) replacing a normal 8. Other chromosome losses, gains and markers are the same (the monosomy 15 is random loss). This cell line had no DMs. The inset (from another metaphase) illustrates the abnormal banding pattern of the 8q⁺ distal to q24. The additional material could not be identified as a normal chromosome segment.

material and of the previously published data, our karyotype of the 1978 cells (Fig. 1) is consistent with that determined by Gallagher *et al.*¹³ at passage 35 of the HL-60 cell line, although this conclusion requires some reinterpretation of the published karyotype (including the putative monosomy 8).

The HL-60 specimens frozen at the Wistar Institute in 1979 (79A, 79B) also had the same karyotype except for one significant change: a normal chromosome 8 was replaced by an 8q⁺ chromosome with an abnormal banding pattern (Fig. 2). This marker, slightly larger than a group B chromosome, had the characteristics of a normal chromosome 8 to band q24 on the long arm, followed by a banded segment that did not appear to be either a duplication of a portion of 8q or a segment of another chromosome. As with the 1978 cells, 5–10% of the metaphases in this specimen were tetraploid, but DMs were not observed in any of the cells. When chromosome studies were done on HL-60 cells that have been passaged continuously at the Wistar Institute since their arrival in 1978 (and that are known to have become tetraploid in 1981), the karyotype proved to be a tetraploid version of the 1979 cells, with two copies of the 8q⁺ chromosome, in addition to two normal chromosomes 8, and no DMs (Table 1).

At the time of the cytogenetic studies on the HL-60-79B specimen, these cells were also fused with P3Bu4 mouse myeloma cells¹⁷. The derived hybrid clones were subsequently analysed for retained human chromosomes¹⁸, and for the estimated frequency of human *c-myc* DNA sequences. Some of the hybrid clones (CL8 and 12) that expressed human glutathione reductase (a marker for chromosome 8) contained a low copy number of the oncogene, while others (CL26 and 33) contained many copies of the oncogene. As indicated in Table 1 and Fig. 3, the two clones retaining the 8q⁺ with the abnormal banding region (CL26, CL33) showed elevated levels of *c-myc* (estimated at 10 and 16 copies per cell, respectively), as did the various specimens of the parental HL-60 cell line studied (36 copies per cell). Those hybrid clones that retained only a normal chromosome 8 (CL8, CL12) had very low levels of *c-myc*, and clone 30, which had neither a normal chromosome 8 nor the 8q⁺, had no detectable human *c-myc* sequences, although several other human chromosomes were retained. DMs were not observed in the hybrid clones.

We have examined the chromosomes of HL-60-79B cells by the *in situ* hybridization technique using a *myc* cDNA probe (Ryc 7.4) (ref. 22). Thirty per cent of the grains located over

Table 1 Correlation between amplification of human *c-myc* gene and double minutes or 8q⁺ chromosome with abnormal banding region in HL-60 cells and derived hybrid clones

Cells	No. of chromosomes	No. of normal chromosomes 8	No. of 8q ⁺ chromosomes	Double minutes	No. of copies of human <i>c-myc</i>
HL-60-78B	44–45	2	0	Yes	20–40
HL-60-78A	44–45	2	0	Yes	20–40
HL-60-78C	44–45	2	0	Yes	20–40
HL-60-79A	44–45	1	1	No	Not done
HL-60-79B	44–45	1	1	No	20–40
Hybrid CL8	80–100	1 (+)*	0	No	0.1
Hybrid CL12	80–100	1 (++)	0	No	0.5
Hybrid CL26	80–100	1 (+)	1 (++)*	No	10
Hybrid CL30	80–100	0	0	No	0
Hybrid CL33	80–100	1 (++)	1 (++)	No	16
HL-60	Tetraploid	2	2	No	Not done

Cells designated 78A, B, C and 79A, B were frozen at the NCI (78B) or at the Wistar Institute (78A, 78C, 79A, 79B) in 1978 or 1979 and thawed shortly before the present study. Cells designated only HL-60 were received from the NCI in 1978 and have since been carried continuously at the Wistar Institute; they became predominantly tetraploid in 1981. Cells prefixed by 'CL' are clones derived from hybridization of HL-60-79B with P3Bu4 mouse myeloma cells¹⁷ at the time the HL-60-79B karyotype was determined. The clones were analysed for retained human chromosomes by combined trypsin–Giemsa and G-11 banding¹⁸. Copy numbers were estimated from densitometric scanning of Southern blots using as an internal standard the Simian virus 40-transformed human fibroblast line PAF (arbitrarily assigned a value of 1.0).

* Frequency of metaphases with relevant chromosomes (8 or 8q⁺) in hybrid clones: +, 10–30%; ++, 30–60%.

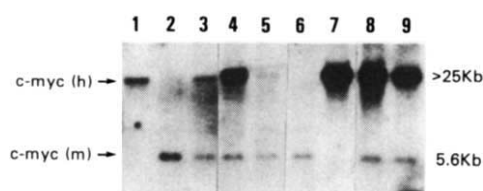


Fig. 3 Southern blotting analysis of DNA from parental and mouse (P3Bu4) $\times$ human (HL-60-79B) hybrids. Following *Bam*HI digestion and agarose gel electrophoresis, the DNA was blotted to a nitrocellulose filter and hybridized with 32 P-labelled Ryc 7.4 human *c-myc* cDNA²¹. Lane 1, simian virus 40-transformed PAF human fibroblast DNA; lane 2, P3Bu4 mouse myeloma DNA; lane 3, hybrid clone 12 DNA; lane 4, hybrid clone 77B10 DNA (no chromosome data); lane 5, hybrid clone 8 DNA; lane 6, hybrid clone 30 DNA; lane 7, HL-60-79B DNA; lane 8, hybrid clone 33 DNA; lane 9, hybrid clone 26 DNA. (h), human; (m), mouse.

chromosomes in these preparations were found, frequently in clusters, over the distal half of the long arm of the 8q⁺ marker chromosome which was identified by its morphology and G-band pattern (Fig. 4). Such heavy, site-specific labelling indicates the presence of multiple copies of the *c-myc* sequence within this chromosomal region.

These cytogenic findings suggest that during the continuous passage of the HL-60 cells at the Wistar Institute there has been a change in an otherwise stable karyotype, with the acquisition of an ABR involving the terminal portion of the long arm of chromosome 8, and concurrent loss of DMs. There is increasing evidence that DMs, ABRs and HSRs represent interchangeable forms of gene amplification units, with those incorporated into chromosomes (HSRs, ABRs) being somewhat more stable, as the DMs, lacking centromeres, may be lost during cell division^{5-7,19}. It has also been suggested that in neoplastic cells such structures may involve genes important in tumour development^{5,6,19}. To date, however, no oncogene or oncogene product has been specifically identified in association with an ABR, although *c-myc* amplification has been related to an HSR on the X chromosome in the human tumour cell line C010320 (ref. 23).

The present results provide another opportunity to make such an association. Both in the parental HL-60 cells and in the derived mouse-human hybrids, increased copies of the *myc* gene were found consistently related to the presence either of DMs or of the 8q⁺ chromosome with the putative ABR (Table 1). Although one cannot rule out the possibility that these structures also involve amplification of other genes²⁰, our data indicate that they represent *c-myc* amplification, and perhaps had a significant role in the pathogenesis of the acute promyelocytic leukaemia from which the HL-60 cell line was derived. The DMs were apparently present in the leukaemic cells *in vivo*¹³, and recent evidence both in the human Burkitt's lymphoma and in murine plasmacytomas suggests that mammalian analogues of the viral *myc* gene may be important in the development of lymphoid tumours^{14-16,18}. In those cases, chromosomal translocation of the *myc* gene to a location adjacent to an immunoglobulin gene may lead to *myc* activation^{18,21}, and this finding in two different species strongly supports a role for this oncogene in both mouse and human B-cell neoplasia.

Furthermore, the specific chromosome change that we have found in the HL-60 cells now carried at the Wistar Institute is consistent with our findings that it reflects the site of *myc* gene amplification. The putative ABR involves the terminal portion of 8q, the recently mapped location of the *myc* gene¹⁴⁻¹⁶. It appeared at the same time that DMs disappeared from the cells, with no other change in the karyotype; and the presence of an ABR rather than a HSR fits the 10-40-fold amplification of *c-myc* that was found in this and previous studies of HL-60 cells^{11,12}.

Taken together, the present data provide strong, if indirect, evidence that *c-myc* gene amplification in the HL-60 leukaemia

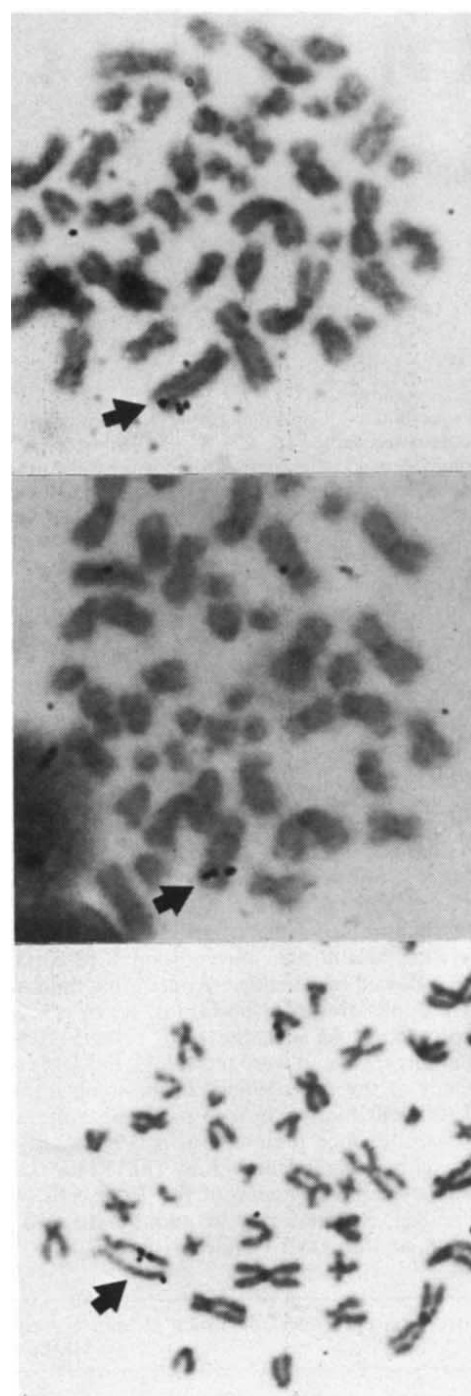


Fig. 4 Three representative autoradiographs from *in situ* hybridization of *c-myc* probe to metaphase chromosome preparations from HL-60-79B cells. Heavy arrows indicate the 8q⁺ marker chromosome with clusters of grains on the distal long arm. Air-dried chromosome preparations were initially treated with RNase and then denatured in a 70% formamide (v/v) 2 $\times$ SSC solution, pH 7.0, at 70 $^{\circ}$ C for 2 min. *myc* cDNA was radioactively labelled with 3 H-dCTP and 3 H-dGTP (NEN) to a specific activity of 4×10^7 c.p.m. per μ g DNA. Radioactively labelled *c-myc* cDNA diluted to a final concentration of 0.07 μ g ml⁻¹, was hybridized to denatured chromosomes for 16 h at 37 $^{\circ}$ C. After removal of unhybridized DNA by a series of washings, slides were dipped in nuclear track emulsion (Kodak NTB2), stored in lightproof containers at 4 $^{\circ}$ C and developed 14-20 days later. After staining the slides with 0.25% Wright's stain, metaphases of good quality were examined for the presence of silver grains overlying individual chromosomes.

line was originally in the form of DMs, and now, in the cells carried at the Wistar Institute, is present as an ABR on 8q.

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Rat *c-myc* oncogene is located on chromosome 7 and rearranges in immunocytomas with t(6;7) chromosomal translocation

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Two B-cell-derived tumours, human Burkitt's lymphoma (BL) and murine plasmacytoma (MPC), are regularly associated with a distinctive form of chromosomal translocation (for reviews see refs 1, 2). In BL, the distal portion of chromosome 8 breaks off and is transposed, in most cases, to chromosome 14, known to carry the immunoglobulin heavy-chain locus³. In about 5% of the cases the same distal part of the chromosome 8 has moved to either chromosome 2 or 22, to the neighbourhood of the κ or the λ locus, respectively^{4,5}. In MPC the distal region of chromosome 15 is transposed to the chromosome 12, known to carry the immunoglobulin heavy-chain locus⁶, or enters into reciprocal exchange with the κ locus-carrying chromosome 6 (ref. 7). Several laboratories have located *c-myc*, the cellular homologue of the MC29 retroviral oncogene *v-myc*, to human chromosome 8 (refs 8-10) and mouse chromosome 15 (refs 11-13). It has also been shown that the BL- and MPC-associated translocations remove the *c-myc* gene from its original site and transpose it into or close to one of the immunoglobulin gene clusters^{8,14-16}. In view of the above findings we also looked for possible involvement of the *c-myc* gene in a B-cell-derived tumour of a third species, the rat. Rat immunocytomas of spontaneous origin carry a reciprocal translocation between chromosomes 6 and 7 (ref. 17). Here we have localized the *c-myc* locus to chromosome 7 of the rat. Moreover, we have found that the *c-myc* gene was rearranged in four of five immunocytomas carrying the characteristic chromosomal translocation.

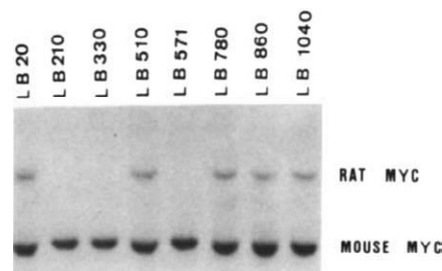


Fig. 1 Restriction enzyme analysis of DNA obtained from rat-mouse somatic cell hybrids¹⁹. High molecular weight DNA was digested to completion with *Hind*III as recommended by the manufacturer (Boehringer Mannheim), subjected to electrophoresis in 0.8% agarose and transferred to nitrocellulose filter (Schleicher & Schuell BA85) as described by Southern²². Hybridization was carried out with the nick-translated²³ *Eco*RI-*Cl*aI fragment of the cloned human *c-myc* locus²¹ as described by Wahl *et al.*²⁴. Filters were washed in 0.2×SSC at 65 °C for 2×30 min.

The LOU/Wsl inbred rat strain develops a high frequency of spontaneous immunocytomas originating from the ileocaecal nodes¹⁸. Our cytogenetics group has found a consistent translocation of the long arm of chromosome 7 to the telomeric end of chromosome 6 in these tumours¹⁷. Banding homologies suggested that this translocation may be analogous to the MPC-associated typical (12;15) translocation. If true, this would suggest a common mechanism of plasmacytomagenesis at the molecular level in both species.

As a first step towards a study of the rat (6;7) translocation at the DNA level, we have localized *c-myc* on the chromosome map of the rat and tested it for gene rearrangement in the immunocytomas. For mapping the *c-myc* gene we screened restriction endonuclease-digested DNAs from a series of somatic cell hybrids derived from the fusion of normal rat hepatocytes with mouse hepatoma cells¹⁹. The hybrid segregated rat chromosomes while maintaining a full complement of mouse chromosomes²⁰. Hybrids containing different combinations of rat chromosomes were tested for the presence of *c-myc* sequences. Digestion of rat liver DNA with the restriction endonuclease *Hind*III produced a fragment that hybridized with the 32P-labelled *Eco*RI-*Cl*aI fragment (pMC41-3RC) of the cloned human *c-myc*²¹. Mouse DNA also yielded a single fragment that reacted with this DNA probe, but the rat *c-myc* oncogene could be readily distinguished from its murine homologue. The rat-derived *c-myc*-specific *Hind*III fragment migrated as a 12.5-kilobase (kb) band while the mouse counterpart appeared as a 5.6-kb band (Fig. 1).

This species-specific restriction fragment polymorphism permitted us to detect the rat *c-myc* gene in the mouse background by Southern blots. As shown in Fig. 1 and Table 1, the hybrid line LB210 and 571 lacked rat chromosomes 6 and 7 and

Table 1 Rat-mouse hybrid cell panel tested for the presence of rat *c-myc* gene

Hybrid cell line	Rat chromosome retained	Rat <i>c-myc</i>	Rat chr. 7
LB20	2, 6, 7, 13, 16, 17, 18, 19, 20, X	+	+
LB210	4, 13, 14, 16, 18, X	-	-
LB330	2, 4, 6, 10, 12, 16, X	-	-
LB510	1, 2, 3, 4, 5, 7, 12, 13, 14, 15, 16, 17, 18, 20, X	+	+
LB571	2, 3, 4, 11, 12, X	-	-
LB780	2, 3, 4, 5, 7, 10, 11, 13, 17, 18, 19, X	+	+
LB860	2, 3, 4, 7, 9, 11, 12, 13, 15, 16, 17, 18, 20, X	+	+
LB1040	3, 4, 5, 6, 7, 11, 12, 15, 16, 18, 20, X	+	+

Segregation of rat chromosomes and rat *c-myc*-specific hybridization in a panel of rat-mouse somatic cell hybrids. Each cell population used for DNA extraction was subjected to detailed karyotype analyses.

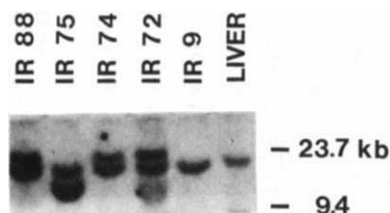


Fig. 2 Restriction enzyme analysis of high molecular weight DNAs obtained from five spontaneous rat immunocytomas and from rat liver. Digestion with restriction enzyme *Eco*RI was done as recommended by the supplier (Boehringer Mannheim). Blotting, hybridization and washing conditions were as described in Fig. 1 legend.

contained no rat *c-myc*-specific fragment. The hybrids LB510, LB860, LB780, which contained chromosome 7 but not 6, yielded *myc*-specific fragments corresponding to the mouse and rat *c-myc* sequences, respectively. A rat-specific *myc* fragment was not detectable in LB330, which contained chromosome 6 but lacked chromosome 7. These results indicated that sequences homologous to the rat *c-myc* gene segregate only with rat chromosome 7. The hybridization pattern was discordant with the presence of any other rat chromosome. Chromosome 8 was not present in any of the hybrids and could thus not be tested. With this limitation we have assigned *c-myc* to rat chromosome 7. Our conclusion is in line with the extensive banding homologies between the *c-myc*-carrying mouse chromosome 15 and rat chromosome 7¹⁷.

As a first approach to the question of whether the (6;7) translocation of rat immunocytomas involved the *c-myc* oncogene, we have compared the *myc*-specific hybridization pattern of restriction enzyme-digested total genomic DNA obtained from normal and tumour cells. Four of the five tumour lines examined (IR72, IR74, IR75, IR88) showed a second rearranged *c-myc* fragment in addition to the normal germ-line sequence (Fig. 2). The rearranged fragment was larger (22 kb) than the normal 16.5-kb *Eco*RI fragment in three tumours and was shorter (10 kb) in one, IR75. Rat liver DNA contained only the 16.5-kb germ-line *Eco*RI fragment (Fig. 2). The size difference among the rearranged *c-myc* fragments suggests heterogeneity in the location of the breakpoint. These findings are similar to the situation in BL and MPC where novel *myc*-specific fragments were often present, in addition to the normal-sized fragments in DNAs derived from tumours containing the characteristic translocation^{8,14-16}. In conclusion, our results show that the *c-myc* locus of rat resides on chromosome 7, which is regularly involved in a plasmacytoma-associated chromosomal translocation. We have also found that the 16.5-kb long *Eco*RI fragment which carries the normal *c-myc* locus was rearranged in four of the five immunocytomas tested, suggesting that the *c-myc* locus is near the translocation breakpoint on chromosome 7.

In human BL and MPC, the chromosomal translocation appears to separate one of the two *c-myc* homologues from its normal regulatory elements and to bring it into the regulatory field of an immunoglobulin gene region. In MPC the typical, that is most frequent, 12;15 translocation links the *c-myc* locus of chromosome 15 to the immunoglobulin heavy-chain locus of chromosome 12. In most cases it faces the *C_α* region 5' to 5' (refs 8, 14-16). In the rat, the chromosomal localization of the immunoglobulin genes is unknown. On the basis of a comparison between the G-banding patterns of mouse chromosome 12 and rat chromosome 6, our cytogenetics group has suggested that at least the distal part of rat chromosome 6 is homologous to the distal region of chromosome 12 in the mouse¹⁷. The bands involved in the plasmacytoma-associated translocation were indistinguishable in the two species. The likely possibility that the *Igh* locus of rat is localized on the distal part of chromosome 6 is presently under study.

The regular occurrence of *c-myc* rearrangement in a B-cell-derived tumour of a third species, the rat, gives further support

to the postulate based on earlier findings on murine plasmacytoma and human Burkitt's lymphoma that specific chromosomal translocations regularly associated with these tumours have an important role in their origin.

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Electrostatic interactions in the assembly of haemoglobin

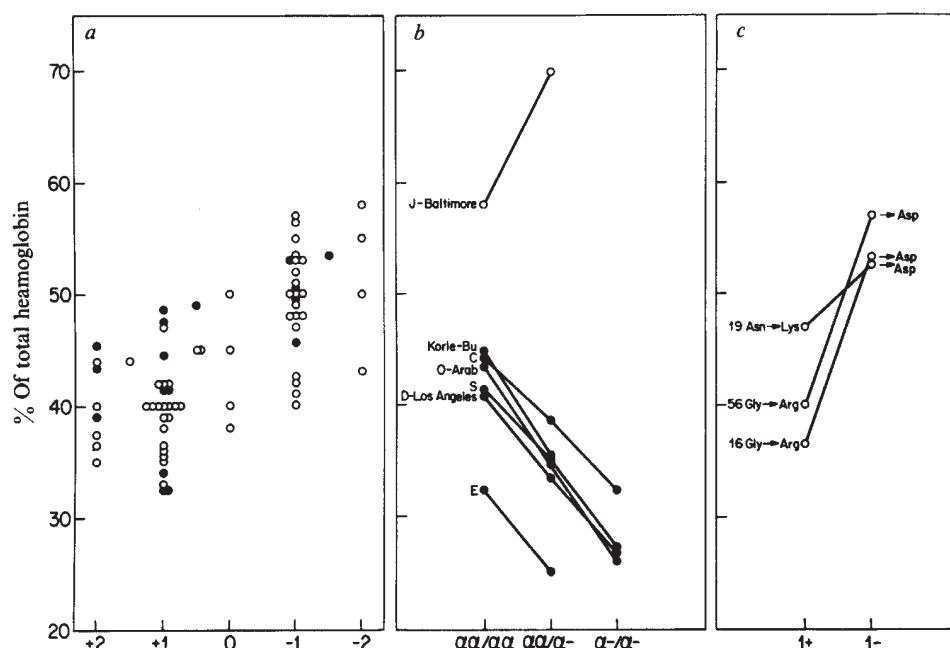
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Haemoglobin is the prototype of an allosteric protein in which cooperative behaviour depends on interaction between unlike subunits¹⁻³. Here we present haematological and biochemical evidence that electrostatic interactions are an important determinant of haemoglobin assembly. Individuals heterozygous for positively charged β -globin variants have a significantly lower proportion of abnormal haemoglobin than those with negatively charged variants. Moreover, these differences become more pronounced when α -thalassaemia is also present. Kinetic experiments using isolated chains indicate that the rate of assembly of the heterotetramer is influenced by alterations in surface charge. A simple electrostatic model is proposed in an attempt to explain these haematological and experimental findings.

Hb A ($\alpha_2^A \beta_2^A$) comprises over 90% of haemoglobin in normal human red cells. The polypeptide chains are encoded on two β -globin genes (one from each parent). Thus, individuals heterozygous for a β -chain variant would be expected to have approximately equal amounts of normal and variant haemoglobins. A markedly low proportion of β -chain variant (<25%) is encountered under two circumstances. Some mutations result in unstable haemoglobin variants which undergo increased catabolism before and after emergence of red cells from the bone marrow^{4,5}. Second, a few variants such as Hb Lepore and Hb Vicksburg are synthesized at a much lower rate than the

Fig. 1 *a*, Effect of charge on the proportion of abnormal haemoglobin in individuals heterozygous for 72 stable β -globin variants collected from the literature. Each data point (○) represents a mean value for a given variant. Substitutions involving a histidine residue were scored as a change of $\frac{1}{2}$ charge. The '−1' group differs significantly from the '+1' group ($P < 0.001$) and from the '0' group ($P < 0.05$). *b*, Effect of α -thalassaemia on the proportion of six positively charged β -chain variants and of one negatively charged variant (Hb J Baltimore). *c*, Comparison of three pairs of β -chain variants having either a positive or a negative charge substitution at the same site. These data could be influenced by analytical artefacts owing to overlap of minor haemoglobin components of Hb A with negatively charged variants. However, these components comprise less than 8% of the total haemoglobin. Moreover, the differences shown in *a* were confirmed in a smaller group of samples analysed by Huisman⁷ using a high resolution chromatographic system which readily separates major and minor components (●). Because of these minor components along with Hbs A₂ the sum of Hb A and the variant haemoglobin comprise about 90% of the total haemoglobin.

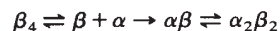


normal β -chain and therefore produce a thalassaemic phenotype. However, the great majority of β -chain variants investigated are synthesized at the same rate as β^A and have normal stability. Nevertheless, even among these variants, the proportion of normal and abnormal haemoglobins in heterozygotes varies widely (Fig. 1*a*). Many commonly encountered β -chain haemoglobin variants, such as Hbs S, C, E and D-Los Angeles, comprise significantly less than half of the total haemoglobin. Equally puzzling is the fact that some β -chain variants exceed Hb A.

Figure 1*a* shows a comparison of all the stable β -chain variants for which information is available. Unstable variants were excluded from this analysis along with those having substitutions at the $\alpha_1\beta_1$ or $\alpha_1\beta_2$ interfaces, as structural alterations at these sites could directly affect the rate of subunit assembly. As Fig. 1*a* shows, the positively charged variants like Hb S, D, C and E all comprise significantly less than half of the total haemoglobin in heterozygotes and are reduced further in the presence of α -thalassaemia (Fig. 1*b*)^{6,7}. In contrast, many of the negatively charged variants are present in amounts exceeding that of Hb A. In one instance where a negatively charged variant (Hb J-Baltimore) has been encountered in an individual with α -thalassaemia, the proportion of the variant haemoglobin is further increased (Fig. 1*b*). There are three sites on the β -chain in which both a negatively charged and a positively charged substitution have been encountered. In each case the negatively charged variant is significantly more abundant than the corresponding positively charged variant (Fig. 1*c*). Virtually all the amino acid substitutions included in this analysis are located at the surface of the haemoglobin molecule. Neither the position of the substitution nor its distance from the subunit interfaces has any detectable influence on the distribution of haemoglobins.

This analysis of the β -chain variants suggests that alterations in surface charge could contribute to different rates of assembly of the haemoglobin tetramer. In the developing erythroblast, newly translated α and β subunits bind haem groups and then combine irreversibly to form a stable $\alpha\beta$ dimer. However, haem-intact β -chains also aggregate with each other to form tetramers (β_4). Stability of β_4 is markedly dependent on solvent conditions⁸ as well as on oxygenation⁹. Both phosphate and hydrogen ions stabilize the β_4 tetramer^{10,11}. The extent of aggregation of β -chains in the developing erythroblast is unknown. In contrast, haem-intact α -chains only aggregate at

high concentrations ($K_{2,1} = 8.4 \times 10^{-3} \text{ M}$)¹². Thus, in the erythroblast the α subunit is almost certainly a monomer. Accordingly, the following reactions can be written for the assembly of the haemoglobin tetramer in the developing erythroblast:



To test the effect of charge on the assembly of haemoglobin, haem-intact subunits were prepared by a modification¹³ of the method of Bucci and Fronticelli¹⁴. Equimolar mixtures of α - and β -chains result in complete recombination to $\alpha_2\beta_2$. Moreover, the oxygen binding of this reconstituted haemoglobin is indistinguishable from that of native Hb A. Only the oxy form was used in these experiments because in the erythroid precursor cell, the haemoglobin subunits would be fully oxygenated owing to their high oxygen affinity. We have used changes in visible spectra^{15,16} to monitor the dissociation of β_4 into monomers and the subsequent combination of α and β monomers to form the $\alpha\beta$ dimer^{17,18}. No absorbance change accompanies the combination of $\alpha\beta$ dimers to form the oxygenated tetramer^{16,17}. In 0.1 M potassium phosphate buffer, pH 7.0 and 20 °C, β^A -haem chains are almost entirely (>95%) tetrameric ($K_{4,1} = 1.4 \times 10^{-19} \text{ M}^3$) (M. Farmer and G. K. Ackers, personal communication). When equivalent concentrations (on a haem basis) of oxygenated α and β subunits were mixed, the reaction followed first order kinetics. In these experimental conditions, the dissociation of oxygenated β_4 -chain tetramers was the rate limiting step in the overall assembly of the $\alpha_2\beta_2$ tetramer. As shown in Fig. 2, the rates of dissociation of the β_4^A , β_4^S (6 Glu $\rightarrow$ Val) and $\beta_4^{\text{N-Baltimore}}$ (95 Lys $\rightarrow$ Glu) were 0.047, 0.029 and 0.11 min^{-1} , respectively, and as expected for a first order process, were independent of concentration (30–90 μM). Thus, the β_4^A -chain tetramer dissociates 1.5-fold more rapidly than β_4^S and 2.3-fold less rapidly than $\beta_4^{\text{N-Baltimore}}$.

The $\alpha + \beta \rightarrow \alpha\beta$ reaction is more difficult to monitor by direct kinetic measurements. Chain competition experiments provide an alternative approach. When a limiting amount of α -chain is added to an equimolar mixture of β^A and β^S chains, less Hb S is formed than Hb A (refs 19–21). This result pertains in solvent conditions where the β -chains are predominantly monomeric²¹. Furthermore, as the relative amount of α -chain is decreased, even less Hb S is formed, relative to Hb A. This experiment is a close parallel to the clinical situation in which a β -chain variant coexists with α -thalassaemia (Fig. 1*b*). Thus, the presence of

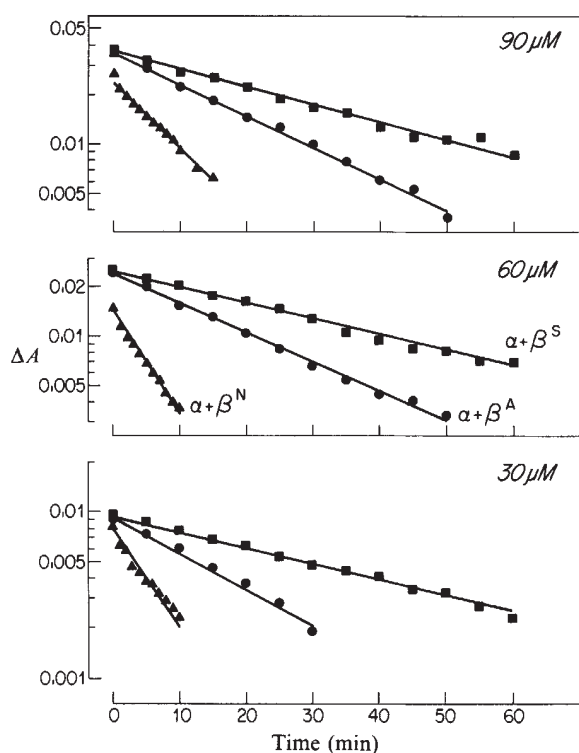


Fig. 2 Semi-logarithmic plots of time courses following the mixing of equivalent concentrations of α - and β -chains (30, 60, 90 μ M in haem) in 0.1 M potassium phosphate, pH 7.0, 20 °C. The reaction was monitored at 582.5 nm where there is maximal difference in the visible spectra between oxyhaemoglobin and its constituent chains¹⁷.

α -thalassaemia, by virtue of limiting production of α -chains, enhances the competition between normal and variant β -chains.

These experimental observations on subunit assembly can be explained by a simple model based on the role of electrostatics in the binding of haemoglobin subunits. The subunits of Hb A differ markedly in charge: β_4^A has an isoelectric point of about 6.8 while α -chains have an isoelectric point of about 8.1 (ref. 21). It is likely that both the dissociation of β_4 into β monomer and the association of α and β monomer to form the $\alpha\beta$ dimer are influenced by electrostatic effects. The enhanced dissociation of the more negatively charged $\beta_4^{N-Baltimore}$ and the decreased dissociation of the less negatively charged β_4^S probably reflect relative electrostatic repulsion and attraction, respectively. Moreover, positively charged α -chains would be expected to combine less readily with positively charged β -chains such as β^S or β^C and more readily with negatively charged β -chains such as $\beta^{N-Baltimore}$.

The available haematological data shown in Fig. 1 are consistent with the above electrostatic model of haemoglobin assembly. This scheme has clinical ramifications. Differences in rates of assembly explain not only the low proportion of Hb S in sickle trait (AS) but also the higher proportion of Hb S in sickle-C (SC) disease. The prominent clinical manifestations of the disease and their absence in sickle trait can be attributed in part to differences in the intracellular content of Hb S²³. This model also provides an explanation for differences in the level of Hb A₂ that accompany certain haematological disorders. Hb A₂ is decreased in various types of α -thalassaemia as well as in two acquired disorders, iron deficiency and sideroblastic anaemias, in which there is also a relative deficiency of α -chain synthesis. It is likely that a deficiency of α -chain production brings out the competition between β^A and the more positively charged δ -chains. As the model predicts, the δ -chains would be at a disadvantage and consequently less Hb A₂ would be formed.

Electrostatic interactions are likely to contribute to the assembly of other multi-subunit proteins. Like haemo-

globin²⁴⁻²⁹, assembled proteins may be considerably more resistant to proteolytic digestion than their free subunits. The rapidity with which subunits bind to one another could influence the turnover and distribution of oligomeric proteins.

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Absence of cooperative haemoglobin-oxygen binding in *Sphenodon*, a reptilian relict from the Triassic

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It is generally accepted that the sigmoidal nature of the haemoglobin-oxygen dissociation curve (ODC) is necessary for efficient oxygen transport in terrestrial vertebrates because it allows large volumes of oxygen to be bound or released for relatively small changes in the partial pressure of oxygen (P_{O_2}) in the blood. Furthermore, the amount of oxygen to tissues is increased by hydrogen ions produced from the dissociation of carbon dioxide in solution^{1,2}. The generality of these key features of cooperative oxygen binding and the Bohr effect holds for reptiles, birds and mammals³⁻⁷, including representatives with special respiratory requirements for diving⁸⁻¹¹, burrowing^{12,13} and living at high altitude^{14,15}. *Sphenodon punctatus* is the sole surviving representative of the ancient order of 'beakhead' reptiles (order Rhynchocephalia) which were once widely distributed during the Triassic period before the spectacular radiation of dinosaur faunas. We have now investigated the oxygen transporting properties of blood from *Sphenodon* and find that the ODC is hyperbolic, with a high affinity for oxygen and very small Bohr effect. This combination of characteristics is unique among terrestrial vertebrates and accords with a low demand for oxygen and limited scope for aerobic activity.

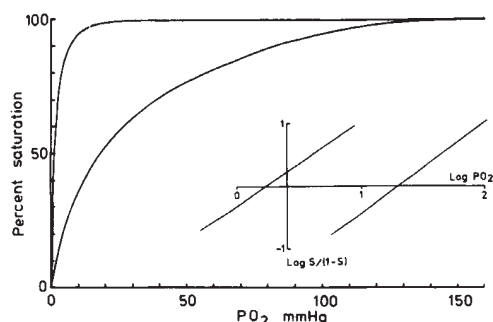


Fig. 1 *Sphenodon* oxygen dissociation curves. Stripped haemolysate at pH 7.5 in 0.05 M Tris buffer and 100 mM NaCl (left) and whole blood at pH 7.6 (right) at 10 °C. Continuous curves were registered from a Hemoscan O₂ Dissociation Analyzer (Aminco) with modifications to reduce gas flow and leakage³³. The HPLC system (Waters Associates) consisted of solvent delivery pumps 6000A and M-45. Peptide separation used a Radial-Pak μ Bondapak C₁₈ cartridge (10 μ m $\times$ 8 mm $\times$ 100 mm) radially compressed in a Z-module. Solvent system: 0.3% (v/v) trifluoroacetic acid and 80% (v/v) acetonitrile/0.1% (v/v) trifluoroacetic acid. Wösthoff gas mixing pumps (Bochum) provided equilibration gases containing CO₂ for whole blood analysis and for pH measurement of tonometered blood in a Radiometer BMS-2 apparatus. Haemolysate was stripped of organic phosphate by passage through a 50 $\times$ 2.5 cm Sephadex column³⁴ and used at a final haemoprotein concentration of 150 μ M. The inset shows Hill plots of the log-transformed data used to express the degree of cooperativity by the coefficient n , equal to the slope. Methaemoglobin was estimated in whole blood and in Hb solution³⁵ after equilibrium measurements and did not exceed 5%.

Sphenodon is now limited in distribution to a few offshore islands of New Zealand, where it is adapted to nocturnal conditions in a cool-temperate climate. Accordingly, it has a marked cryophilic preference with a preferred activity range of 18–22 °C^{16,17}. With special permission from the Wildlife Service of New Zealand, we collected heparinized blood specimens by caudal venepuncture from eight healthy reptiles (350–680 g) brought to the mainland.

Fresh blood was analysed without storage or delay. Microhaematocrit and haemoglobin (Hb) concentrations were measured¹⁸ with the added step of centrifuging the cyanmethaemoglobin solutions to remove nuclear debris. Haematocrit was $24.5 \pm 3.6\%$ and similar to values obtained from other reptiles⁶. Haemoglobin concentrations were lower than those of other reptiles⁶ (59.3 ± 7.7 g l⁻¹), suggesting a low oxygen carrying capacity and an unusually low mean cell haemoglobin concentration of 242 g l⁻¹. This may be related to the exceptionally large size of the erythrocytes, apparently the largest in the reptilian class¹⁹.

Specific red cell trinucleotides were detected by TLC on polyethyleneimine-impregnated cellulose and eluted for quantification using appropriate extinction coefficients^{20,21}. The principal trinucleotide was ATP, occurring at a concentration of 1.2 mol per Hb, with traces of GTP present (<0.2 mol per mol Hb). Neither 2,3-diphosphoglycerate nor inositol polyphosphate could be discerned using enzymatic²² and chromatographic²³ techniques. ATP is also the principal acid-soluble phosphate present in the erythrocytes of other reptiles.²⁴

The whole blood ODC in Fig. 1 is hyperbolic and has a half-saturation P_{50} (P_{50}) of 19 mm Hg. The ODC for haemolysate 'stripped' of allosteric phosphate modulators is shifted well to the left ($P_{50} = 1.8$ mm Hg), but could be restored to 14 mm Hg on addition of ATP to Hb at constant pH in a 9:1 molar ratio. The cooperativity coefficient, n , derived from the slope of the Hill plots in Fig. 1 (inset), was 1.1 for both whole blood and haemolysate, indicating the essential absence of cooperative oxygen binding.

All other terrestrial vertebrates, including reptiles, have sigmoidal binding curves with n values >2.0 at the P_{50} . The absence of cooperativity in the stripped haemolysate suggests that this

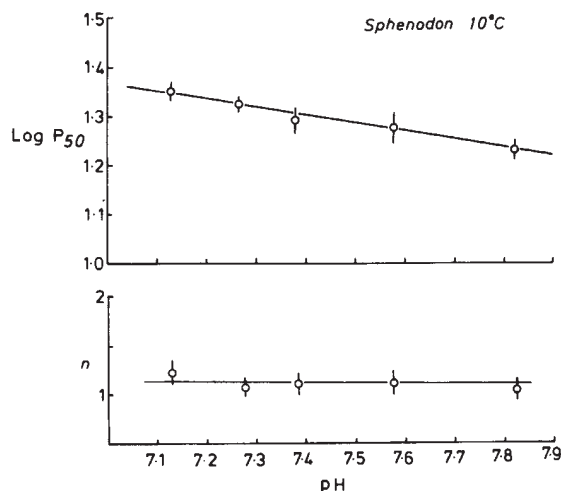


Fig. 2 Bohr plot of whole blood from *Sphenodon* at 10 °C assuming best fit by linear regression, giving a Bohr coefficient, $\Delta \log P_{50} / \Delta \log pH = -0.16$. The lack of sensitivity of Hill's cooperativity coefficient n to pH is shown in the lower portion of the graph. Mean high and low pH n_{50} differences were not statistically significant ($P > 0.1$). pH was varied with CO₂ and measured in a Radiometer BMS-2 apparatus following microtonometry with CO₂ mixes provided by gas mixing pumps. Vertical bars indicate $\pm$ s.d.

feature of the ODC resides in the molecular structure of the haemoglobin. Using standard techniques²⁵, whole haemolysate was separated into three haemoglobin fractions which, when chromatographed on Sephadex G-100, gave tetrameric molecular weights of 64, 500. The composition of the haemolysate and isolated haemoglobins was investigated using HPLC separation (see Fig. 1 legend). Whole haemolysate showed the presence of five distinct globins. Each of the three isolated haemoglobins showed the presence of two types of globin chain. Moreover, back-mixing experiments showed the existence of a common chain in two of the haemoglobins. We envisage the haemoglobins thus represented as Hb I ($\alpha_2\beta_2$), Hb II ($\alpha_2\delta_2$) and Hb III ($\alpha_2\epsilon_2$). Although at present we cannot confirm whether c-globin is α or β type, analogy with other vertebrate systems tends to a separation order suggestive of the β type.

The role of ATP in the control of haemoglobin function has been established for several reptiles, although there are some exceptions. The erythrocytes of crocodiles, for instance, contain low amounts of ATP which are subservient to CO₂ in the regulation of HbO₂ transport¹⁰. Furthermore, the erythrocytes of turtles contain small quantities of inositol polyphosphate, although a possible allosteric role has not been established for these reptiles^{4,24}. It therefore appears that the rhynchocephalian aligns with the Squamata, but that there is an important functional difference. The oxygen affinities of whole blood from squamatus reptiles, including those with low ecritic temperatures and of a similar size to *Sphenodon*, are low, with P_{50} values of over 60 mm Hg (compared with 19 mm Hg) for large iguanids and ananids⁶. This appears to result from the intrinsically lower affinities of reptilian haemoglobins, which are less sensitive to ATP^{26–28} than in *Sphenodon*.

The whole blood ODC was determined over a range of pH to test the sensitivity of the P_{50} to pH. The results are expressed as a Bohr plot in Fig. 2, from which a Bohr coefficient of -0.16 was obtained over the pH range 7.1–7.9. Hill's coefficient n is shown to be essentially independent of pH. The lack of cooperativity, high blood oxygen affinity and small Bohr effect in *Sphenodon* comprise a unique combination of oxygen binding characteristics among air-breathing terrestrial vertebrates.

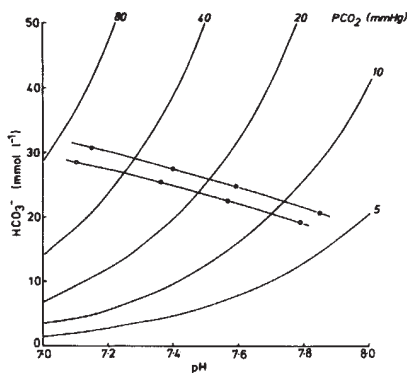


Fig. 3 Plot of $[\text{HCO}_3^-]$ against pH at 10°C with P_{CO_2} isopleths. The buffer lines are for oxygenated and deoxygenated whole blood. Acid-base balance was investigated by equilibrating blood to known CO_2 tension and measuring the pH (Astrup titration). $[\text{HCO}_3^-]$ was calculated from pH and P_{CO_2} with the Henderson-Hasselbach equation using the values of Severinghaus³⁶ for CO_2 solubility and pK at 15°C . The buffer value of these data was estimated to be $\approx 13 \text{ mequiv l}^{-1}$. The Haldane coefficient was calculated as $\Delta[\text{HCO}_3^-] \cdot \text{pH}/\Delta\text{HbO}_2 = -0.22$.

What is the possible significance of the small Bohr effect in *Sphenodon*? One of the few physiological studies with *Sphenodon* indicated that its metabolic rate was markedly lower than that expected from measurements on other similar-sized reptiles (V. T., N. J. Dawson, G. Housley, A. A. Young and R. M. G. W., unpublished). In the absence of direct blood-gas measurements we can only speculate that a low eccentric temperature and slow metabolism would not require a large Bohr effect. This is indirectly supported by the occurrence of a diminished Bohr effect in cold-acclimated tortoises during entry into hibernation²⁷.

As expected from theoretical considerations, the poor proton binding ability of *Sphenodon* blood indicated by the low Bohr coefficient should be matched by a low Haldane coefficient. This equivalence is tested and confirmed by the buffer lines for oxygenated and deoxygenated blood in Fig. 3.

These findings from *Sphenodon* are relevant to the evolutionary scheme of Coates²⁹ favouring low cooperativity and diminished Bohr effects in primitive vertebrates which, with the advent of terrestrial life and increased metabolism in an oxygen-rich environment, permitted exploitation of cooperative oxygen binding and substantial Bohr shifts.

For air-breathing animals, a higher metabolic rate has generally been linked with low oxygen affinity³⁰, in which case larger Bohr effects would be advantageous in bringing about ore efficient unloading of oxygen in tissues which are actively metabolizing and producing acidic wastes. The low affinity of lizard bloods may be due to efficient ventilation and permits large arterio-venous oxygen content differences when demands for oxygen are high, thus sustaining a maximal gradient for oxygen diffusion between blood and tissues.

Reptiles are typically capable of anaerobic bursts for high levels of activity and thereafter return to low resting rates of aerobic metabolism. Blood of low oxygen affinity allows for rapid recovery from oxygen debt in iguanas, where large arterio-venous saturation differences develop during exercise³¹. The *Sphenodon* is capable of fast movements over short distances of a few metres or so, subsequently adopting a cryptically

'freezing' posture if it has not retreated to its normal resting place in a burrow. In its pre-human habitat the species had no natural enemies and would require only occasional bursts of energy to capture invertebrate prey.

Many morphological features of *Sphenodon* do not appear significantly different from rhynchocephalians living some 200 Myr ago. The living species has relatively small lungs with low surface area to volume ratio, suggesting diffusion-limited pulmonary gas exchange³².

We speculate that the primitive lung morphology of *Sphenodon* places constraints on the pulmonary ventilation system which is not compatible with exploiting a low affinity blood having a large Bohr effect and sigmoidal oxygen binding curve. Wood and Lenfant³ have considered poor access to oxygen as a major environmental factor favouring a reduced Bohr effect and high blood oxygen affinity. The *Sphenodon* inhabits roomy burrows which would not appear to limit oxygen uptake—indeed, the burrows are often shared with one of several species of sea birds during the breeding season. We consider an exceptionally low resting metabolic rate to be consistent with a reduced Bohr effect and high blood oxygen affinity.

Considering the wide variety of environmental conditions experienced by reptiles, and the interesting variations in their circulation, Sullivan²⁶ predicted that examination of the oxygen binding properties of their blood might well include the physiological extremes among the terrestrial vertebrates. This has been borne out by a study of the sole survivor of the Rhynchocephalia.

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Electron densities in active galaxies

It is alarming to find a common fallacy concerning the measurement of electron densities in astrophysical conditions appearing twice in the same issue of *Nature*. Both Fairall¹ and Gaskell², argue that the mere appearance of the forbidden lines of O III in a Seyfert galaxy can place an upper limit on the electron density and thus a lower limit to the volume of the emitting gas. This simple argument by itself is spurious. To determine the electron density one must either examine the ratio of two lines whose sensitivity to n_e is different, or use a quantitative measurement of a line-to-continuum ratio.

The range of 'typical' electron densities in Seyfert galaxies has been established through proper modelling and it is unfortunate that simplifications of the arguments have led to the two factually incorrect statements which appear, "As the quenching density is $\sim 10^4 \text{ cm}^{-3}$, we cannot reasonably appeal to higher densities"¹, and "Whenever we see forbidden lines, therefore, we know that the gas density is lower than some critical value"².

Although well known to many astronomers, it seems useful to outline how the population of the metastable level, from which the forbidden transition occurs, varies as n_e increases. At low densities, collisions between electrons and the lower level excite the upper level and are balanced by spontaneous radiative decay—the 'coronal' approximation. Once the collisional de-excitation rate begins to exceed the spontaneous radiative decay rate the population increases less rapidly, but tends to its maximum value, the Boltzmann population, when collisional excitation and de-excitation balance each other. Thus the intensity of a forbidden line increases as the density increases until the Boltzmann limit is reached. The ratio of the intensity of such a line to that of a permitted line, which has a larger transition probability and hence does not reach its Boltzmann limit until much higher densities, will begin to decrease once the metastable level reaches its maximum population. The behaviour with respect to the continuum depends, of course, on how the latter is formed.

The confusions in the descriptions in the literature may have arisen from an incorrect analogy with 'quenching' in laboratory gases³ where different processes may cause the de-excitation and excitation and prevent a true Boltzmann population being reached.

The size of the emitting region in the Seyfert galaxy discussed¹ is therefore open to question until the relative intensities of the lines or continuum emission present are investigated, but the main point of this

comment is to correct the misleading generalizations which have appeared^{1,2}.

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1. Fairall, A. P. *Nature* **304**, 241–243 (1983).
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FAIRALL REPLIES—The value for the upper limit of the electron density, $n_e = 10^6$, used in my letter¹, is simply a standard value widely quoted in the literature (see ref. 2, for example). Whilst I take Jordan's point about the need to determine n_e from sensitive line ratios rather than the appearance of forbidden lines, my value reflects the general consensus and would be acceptable to the experts in the field, one of whom was a referee to my letter.

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1. Fairall, A. P. *Nature* **304**, 241–243 (1983).
2. Tothline, J. E. & Osterbrock, D. E. *Astrophys. J. Lett.* **210**, L117 (1976).

GASKELL REPLIES—While recognizing that the main point of Jordan's comment is to attempt to correct the allegedly misleading generalizations made by Fairall¹ and myself², I must state that the size of the forbidden line emitting region in Fairall 427 is not an open question. If one looks at Fairall's letter¹ one sees that all the line ratios point to low densities. For example, Fairall gives the ratio of the (forbidden) [O III] λ 5,007 line to the (permitted) H λ 4,861 line as 19. At the same time the [O III] λ 4,363/[O III] λ 5,007 ratio, while not explicitly given in the text, is obviously very low (see Fairall's Fig. 1). Quantitative studies of photoionized gases show that such ratios rule out electron densities greater than 10^6 cm^{-3} . Careful reading of Fairall's letter shows that he has not used "the mere appearance of the forbidden O III lines" to place a lower limit on the density. There is no such statement of this sort. In implying that 10^6 cm^{-3} is an upper limit to the electron density Fairall is merely stating what is basic common knowledge in the field of active galaxy research. It is not normal to spell out pendantically the details of such knowledge every time one writes a research paper (and indeed it would waste valuable journal space to do so).

For my News and Views article² I was asked to explain to the general reader of *Nature* why Fairall's claim was so remark-

able and also to explain such technical terms as "Seyfert 2 galaxy" and "forbidden lines". The explanation I gave of the suppression of forbidden lines at high densities is the standard one that has been given for over half a century. It was first given by Henry Norris Russell in 1927 and is quoted by I. S. Bowen in his 1928 paper on forbidden lines in nebulae³. From a pedantic point of view it is, of course, incorrect for us to say that collisions 'prevent' a forbidden transition. A low-probability transition can still be important even at densities orders of magnitude higher than the critical density. This could, for example, be the case for the two-photon 2–1s transition in neutral hydrogen in the dense broad-line region clouds of quasars⁴.

I conclude, by stressing that the density and size of the forbidden line regions of Seyfert galaxies and quasars are two of the best known parameters in the subject area.

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On the half life of ^{138}La

TANAKA AND MASUDA¹ recently have observed $^{138}\text{Ce}/^{142}\text{Ce}$ isotopic abundance ratios which vary linearly with the $^{138}\text{La}/^{142}\text{Ce}$ abundance ratios in samples of ancient rocks. These variations have been interpreted to be the result of *in situ* decay of ^{138}La and thus suggest the potential usefulness of the $^{138}\text{La}/^{138}\text{Ce}$ geochronometer. However, to reconcile the age of the samples inferred from the $^{138}\text{La}/^{138}\text{Ce}$ clock with that obtained from the well-established $^{147}\text{Sm}/^{143}\text{Nd}$ chronometer, Tanaka and Masuda had to use a value of the ^{138}La β -decay partial half life $\sim 16\%$ less than the mean of the published values for this quantity. To justify the use of this shorter half life, Tanaka and Masuda¹ argued that the absorption of atmospheric H_2O and CO_2 by La_2O_3 probably led to overestimates of the amounts of La contained in samples used in ^{138}La half life determinations.

Recently, however, we have reinvestigated the half life and decay scheme of ^{138}La using 99.999% pure La_2O_3 samples². This material was tested for volatile impurities by the manufacturer (J. T. Baker) by heating it to $1,150^\circ\text{C}$ in air for 1 h. Following this procedure, a 0.12% weight loss was observed. After obtaining

this La_2O_3 , two samples were weighed out and then sealed in plastic holders for use in our studies of ^{138}La decay. The La sources were counted using a large volume Ge(Li) detector, and from the measured γ -ray yields, the β -decay and electron capture (EC) decay partial half lives were determined. Subsequent tests on separate samples of this same La_2O_3 showed that when this material is exposed to air for a period of several days, 10–15% weight increases are observed. These weight increases are probably due to the absorption of H_2O and CO_2 as suggested by Tanaka and Masuda¹. However, this process occurs only in material left exposed to air, and thus does not affect the results of our half life measurements.

Our results for the ^{138}La β -decay, EC decay and total half lives are $3.19 \pm 0.22 \times 10^{11}$ yr, $1.58 \pm 0.02 \times 10^{11}$ yr, and $1.06 \pm 0.03 \times 10^{11}$ yr, respectively². These values are in excellent agreement with those recently obtained by Sato and Hirose³. As we have previously discussed², the relatively poor energy resolution of NaI detectors and the nearly unavoidable presence of radioactive impurities in La compounds may have led early investigators to obtain incorrect values for the ^{138}La half lives. Thus one probably should not include the results of such measurements in estimates of the ^{138}La half lives. The only experiments in which Ge(Li) detectors were used and in which attention was paid to the problem of volatile impurities in La_2O_3 samples were ours² and that of Sato and Hirose³. Unfortunately, Sato and Hirose quote only statistical uncertainties in their results³. However, the techniques used in their experiments were very similar to ours. Thus if we assume that their total uncertainty (statistical + systematic) is equal to ours, then we can combine the results of these two experiments. The mean of these two measurements yields a ^{138}La β -decay partial half life of $3.10 \pm 0.15 \times 10^{11}$ yr. Thus the discrepancy between the ages inferred from the $^{138}\text{La}/^{138}\text{Ce}$ and $^{147}\text{Sm}/^{143}\text{Nd}$ chronometers is slightly larger than was originally found by Tanaka and Masuda¹. The origin of this discrepancy remains an open question.

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2. Norman, E. B. & Nelson, M. A. *Phys. Rev. C* **27**, 1321–1324 (1983).
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TANAKA AND MASUDA REPLY—
Norman and Nelson have carefully redetermined the half life of ^{138}La using

a La_2O_3 source¹. They have presented 3.10×10^{11} yr for the partial half life of ^{138}La β -decay by combining their result¹ with that of Sato and Hirose², but still there appears to be some discrepancy between the ages inferred from the ^{138}La – ^{138}Ce and ^{147}Sm – ^{143}Nd chronometers³.

We have re-examined the Ce isotopic composition of the same sample powders as studied previously³, because our technique on the isotopic measurement has recently been improved. New results yield an age of $2,350 \pm 380$ (2 σ) Myr based on the partial decay constant $2.58 \times 10^{-12} \text{ yr}^{-1}$ (half life of 2.69×10^{11} yr). Assuming instead a 13% longer partial half life of 3.10×10^{11} yr ($\lambda\beta^{138}\text{La} = 2.24 \times 10^{-12} \text{ yr}^{-1}$) advanced here by Norman and Nelson, the age comes out to be $2,710 \pm 430$ (2 σ) Myr, which is even more discrepant compared with the ^{147}Sm – ^{143}Nd age ($2,050 \pm 90$ (2 σ) Myr) and ^{87}Rb – ^{87}Sr age ($2,050 \pm 43$ (2 σ) Myr)³. This implies that the inclination of our isochron line obtained for La–Ce systematics is too steep in view of the half life estimated from radioactivity.

If we suppose, on the other hand, that the discrepancy between ^{138}La – ^{138}Ce and ^{147}Sm – ^{143}Nd ages is real, a conceivable explanation is that this discrepancy was caused by alteration effects on the sample examined here. Lanthanum is considered among rare earth elements to be a relatively very mobile element on weathering or alteration. Sometimes the alteration brings about the increase in abundance of light rare earth elements in mid-Atlantic ridge basalts⁴. By addition of the same amounts of La to plagioclase and pyroxene, the $^{138}\text{La}/^{142}\text{Ce}$ ratio of pyroxene increases by a greater factor than that of plagioclase, because the La abundance of pyroxene (0.6–1.0 p.p.m.) is smaller than that of plagioclase (2.3–2.4 p.p.m.)³. The $^{138}\text{La}/^{142}\text{Ce}$ ratio of bulk sample will increase moderately and then the La–Ce isochron will become steeper, giving an apparently older La–Ce age. However, the La abundance is not likely to have been modified, because ^{87}Rb – ^{87}Sr and ^{147}Sm – ^{143}Nd ages agree despite the fact that Rb and Sr are considered to be more mobile elements than La on weathering or alteration.

There are several kinds of uncertainties which may have effects on the absolute age under consideration: (1) isotopic abundance of ^{138}La and ^{138}Ce ; (2) elemental abundances of La and Ce; (3) decay constant of ^{138}La . Uncertainties of isotopic abundance of ^{138}Ce and of elemental abundances of La and Ce were taken into consideration for age calculation³. Uncertainty of ^{138}La abundance was not considered. This uncertainty is estimated at $\sim 1.3\%$ (ref. 5) and it has an effect both on decay constant and on $^{138}\text{La}/^{142}\text{Ce}$ ratio in age calculation. However, uncertainties of the both terms are mathematically cancelled in the course of the age calculation.

Uncertainties in the decay constant should be evaluated from various sides. However, uncertainties for the most of the decay constants obtained previously (see Table 1 of ref. 3) have been estimated only from counting statistics. For the absolute evaluation and comparison between individual radioisotope clocks, all uncertainties on the procedures must be taken into account. For the accurate quantitative measurement of 789-keV γ ray, especially, which is an emission from excited ^{138}Ce , careful corrections such as detector efficiencies and relative attenuation by self-absorption compared with the standard γ -ray (for example, 1,461-keV γ -ray from ^{40}K) are required. From this standpoint, the 14% (2 σ) uncertainty in γ -decay constant of ^{138}La by Norman and Nelson¹ seems to be the only reliable evaluation. If 10% (2 σ) uncertainty estimated here by Norman and Nelson) uncertainty in the decay constant is taken into account for the age calculation, the resultant La–Ce age of the Bushveld gabbro falls in the range agreeable to the Sm–Nd age within experimental uncertainty. In this sense, we would again like to emphasize that it is essential to establish a precise and reliable value for the decay constant of ^{138}La . La–Ce geochronological studies which compare the Sm–Nd clock for some material older than the Bushveld gabbro such as meteorite will give that refinement.

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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

Drawing the strands together

Gene Mappin

DNA for Beginners. By Israel Rosenfield, Edward Ziff and Borin Van Loon.
Writers & Readers (distributed by W. W. Norton): 1983. Pp.223. Pbk \$6.95.
To be published in the UK in February 1984, price £2.95.

Hi Brid Vygor stood on the bridge of his ship, the *James Dewey Watson*, watching the last preparations for take off. They were destined for the Helix Nebula, 194 giga bases away, with a valuable cargo of allosteric effectors. As he adjusted the supercoils on the massive meiotic drive units, the great ship slowly lifted off. Then, with a surge of 23 megacricks of pure cistrionic energy, she leapt forward. . .

DNA is constantly coming of age but now this is in single figures and as a special mark of celebration we have a guide for beginners in the novel form of a comic book which can be easily read by all nine-year-old scientists. It will be particularly appreciated by many of our colleagues who, up to now, have had to be satisfied with the inferior substitute of the xerox machine. The book covers an enormous range and since its illustrations are, of course, the main part, it has to be said that it is richly and profusely complemented by a text that has just enough spelling mistakes to give the book an authentic flavour. It is replete with many subtle allusions, popular and *recherché*, classical and modern; so many, in fact, that a detailed deconstructionist textual analysis would fill many Ph.D. theses.

We start with a dramatically illustrated page on von Neumann's theory of self-reproducing machines. The robot loading another with an instruction tape (the I component, if I remember correctly) is for the more mature *aficionado*, and younger boys will rapidly turn on but should not take too literally the next picture of the well-dressed chimpanzee threading its somewhat limp offspring with DNA. This is what is known in the trade as a metaphor. The history of genetics opens with a typical cell and a not-so-typical scientist just off the beach at La Jolla whose dim awareness must be due to the rather opaque shades he so elegantly sports. Friedrich Miescher, the discoverer of DNA, has suspiciously loose cervical vertebrae with too many degrees of rotational freedom and Mendel appears undeterred by the Jolly Green Giants and their mutants. Weismann is found climbing back into an egg with most of his soma deleted so he can retain a pair of threateningly large boots. The chimaeric figure of Lamarck bears a striking resemblance to Mrs Thatcher and H.J. Muller progressively acquires more and more of the characters of *Drosophila* with bizarre homoeotic consequences.

And so we pass to the modern era. A somewhat Proustian Griffith shares with Avery the discovery of DNA transformation and — after a short chemical interlude — SHAZAM! enter Watson and Crick. There is a super drawing of Jim but across the page where he appears with Luria there is a strong suggestion of a ventriloquist and his dummy. Alas, the drawings of Francis are poor, but the famous pair appear with the model as Batman and Robin, distinctly labelled so you know who is who. Fred Sanger makes a first appearance (not a good likeness) and Sydney Brenner, depicted as a zoot-suited Mongolian of fractal dimension, is found clutching an extremely tired-looking Francis. The reader is warned that the history is not entirely accurate and is reminded of the extreme improbability of Francois Jacob saying "Rhubarb, Rhubarb" and, later, "Aw Shucks".

The core of the book contains descriptions of the central molecular processes of replication, transcription, translation and genetic regulation, rather well illustrated by machine analogies. I adored the polypeptide chain of machines trundling out of the ribosome factory and then folding up with a SPROING! I am certain sproing theories would get rather short shrift in other pages of this journal. After an account of bacterial genes with the ambling shape of Joshua Lederberg we are with animal cells. The attractive lady on page 111 has a most improbable cytology and I am haunted by that sad and lonely bacterium reluctantly eating two sardines.

Enter genetic engineering with explan-

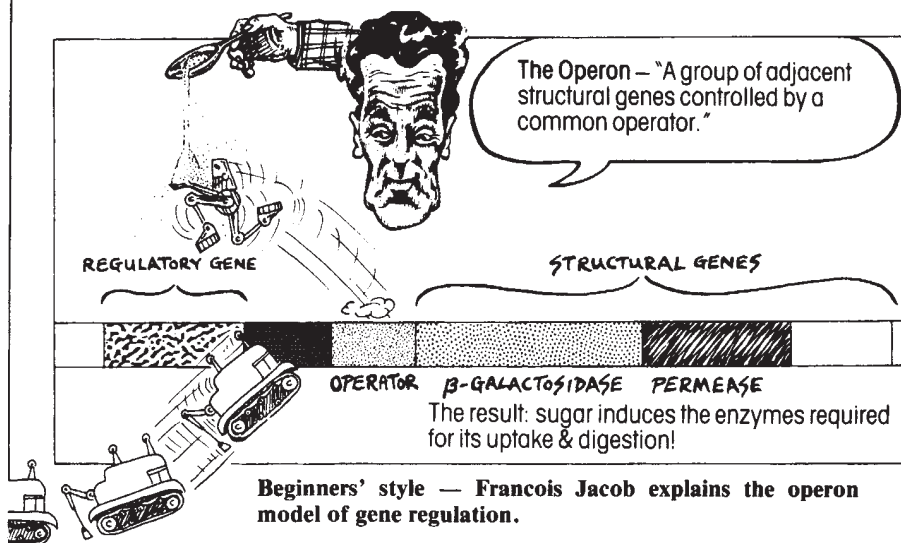
ations of the techniques of cloning and sequencing with scissors, gluepots and other mechanical devices; then eukaryotic transcription, introns, chromatin, Z-DNA and molecular evolution.

We also have the social impacts of DNA. There is a resemblance between the skin of the cloned monster and Wally Gilbert's jacket but that, I am sure, is purely coincidental. It is a marvellous monster and I only wish I could make one. We pass on to the moratorium, the public debate on recombinant DNA, biotechnology and its promised benefits. Finally, there is a discussion on the origins of life with cans of prebiotic soup that look quite hard to open, and another look at evolution and selfish genes.

The postscript, which has no pictures, is very good and will be enjoyed by the older, more serious-minded reader. I find it hard to tell how much this book will be read for instruction and how much for sheer entertainment. A real beginner may still find the subject-matter hard going but the cartoons help and, as popularization, it is to the bland dronings of the glossy pages of popular science magazines as chocolate cream cake is to sliced bread. Read it and enjoy it, and try to give it to your friends before they give it to you. □

. . . as the ship emerged from intracisternal space, Hi Brid Vygor cast his mind back to the first successful Hfr transfer, remembering the stirring words of President T.G. Exon — "Let us now go forward to boldly clone and to joyfully express. . .". He turned to his engineer Hin D III. "Cut", he said, with a faint smile.

The reviewer is a molecular biologist who enjoys a considerable reputation in a somewhat limited circle. He is delighted to affirm that the above should be construed as an advertisement not only for himself but also for his friends who wrote the book.



Natural history and the rogue elephant

A.J. Cain

Dear Lord Rothschild: Birds, Butterflies & History.

By Miriam Rothschild.

Hutchinson/Balaban, Philadelphia
(distributed by ISI Press): 1983.
Pp.398. £14.95, \$29.95.

MIRIAM Rothschild is well-known as a first-class naturalist and excellent writer (witness her part in *Fleas, Flukes and Cuckoos* written jointly with Theresa Clay), and in this remarkable book, she turns her affectionate but deadly perceptiveness on that most extraordinary man her uncle, Lionel Walter, second Baron Rothschild of Tring (1868–1937). I found the book absolutely compulsive reading, sometimes hilariously funny, occasionally genuinely heart-rending, but always fascinating.

It is written for the general intelligent public, not for scientists. This is only reasonable, considering that Lord Rothschild was a local benefactor and employer, an MP, and the recipient of the Balfour Declaration (of intent to support the creation of the State of Israel). In succession to his father, he was the man regarded as the lay head of the Jewish communities of the world. All this as well as one of the greatest collectors of natural history, and the greatest benefactor ever of the British Museum (Natural History) (BM(NH)). The only family activity he did not shine in was banking.

Of the 35 chapters — several devoted to his parents, his brother and that outstanding organizer, his sister-in-law — most deal with Walter Rothschild's scientific development, his collections, his Museum, his curators and his collectors. Four cover his relations with the world Jewish community, the British Government, and the setting up of Israel, one mainly his soldiering in the First World War, one mainly his hopeless failure at banking and the Great Row with his father. His various mistresses and the peeress who blackmailed him so successfully that he had to sell his great collection of birds flit in and out of the chapters as they did during his life.

Miriam Rothschild's last chapter, her summing up, begins:

Walter Rothschild beat the system — in fact he beat two systems — in order to become the best known zoologist of his day and the greatest donor of all time to the British Museum of Natural History. He was particularly inept at

traditional book learning, but he broke loose from the toils of the conventional Victorian education. To the annoyance and irritation of the pedagogues and run-of-the-mill zoologists, he pursued a course of wilful but creative self-instruction. . . . The second system he beat eventually, but for which, nevertheless he had to pay a dreadfully heavy price — namely 18 years of the most creative period of his life, spent at his desk at New Court — was the combined pressures and expectations of his family and the Jewish community.

This is a very fair statement. Walter was a dedicated naturalist and collector, but a man for whom it was impossible to say anything on emotional matters. On impersonal ones he could be relaxed, genial and communicative — and terribly unpredictable. A rogue elephant is the simile Miriam Rothschild uses, with reason.



Walter Rothschild in 1895, at Tring in Hertfordshire, with one of the zebras he broke in himself.

What distinguishes Walter Rothschild as a naturalist is that he not only collected but went on to study his collections in a thoroughly scientific way. He was one of the earliest naturalists (outside Germany) to understand variation in natural populations, its importance for evolution and the necessity for long series as a basis for investigating it. As he wrote in his memorandum on the gift of the Tring Museum to the BM(NH):

My long connection with Zoology has convinced me that the methods of research in systematics — the ultimate object of which is the elucidation of relationship and evolution — require amplification. One point strikes me as particularly important: the systematist is constantly hampered by the fact that the material at hand is not sufficiently large, the conclusions drawn remaining provisional, requiring corroboration by the study of further material. It would be a great advance, and give the systematics of a particular group or order the necessary sound

basis, if of some species at least the material of specimens from the entire area occupied by the species were available in such large numbers that it fully represented the range of variation in each district.

This was the conviction he had acted on for many years, which (apart from a childlike delight in huge specimens) had governed him in building up the enormous collections at Tring. This idea must have aided him in making his excellent choices of his assistants; his curator of vertebrates was Ernst Hartert, and of invertebrates Karl Jordan. Add to this that he spotted Ernst Mayr as an outstanding young ornithologist, and it is clear that he had real insight and judgement. Not only that but both Hartert and Jordan remained at Tring for almost all their working lives, and collaborated extensively both in the collecting and curating, and in publication with Walter Rothschild. The forty-odd volumes of the *Novitates Zoologicae* merit a careful study by a real historian of zoology.

Tragically, it may be impossible ever to write a complete history of science at Tring. Not only were the estate records destroyed by Victor Rothschild's agent, Major Fellows, during the Second World War, not only did Walter destroy most of his own private papers (while characteristically leaving some most indiscreet documents around), but far worse, in the late 1960s, the entomological building was pulled down to make a site for the BM(NH)'s new ornithological department, and the priceless archives of the Museum as it was under Walter Rothschild were simply burnt by the works progress officer, in spite of agonized protests.

Of the curators, Ernst Hartert has a place in the history of both ornithology and systematics generally (see Mayr's *The Growth of Biological Thought* (Harvard University Press, 1982) for an assessment of both him and Jordan). Hartert was already a pioneer in the recognition of geographical as against individual variation when Rothschild attracted him to Tring. The recognition of geographical variation by the use of trinomial names was a feature of the *Novitates* right from the beginning, against the strong opposition of most British systematists. (In this, Britain was well behind both Germany and the United States.) Karl Jordan, however, was by far the greatest brain at Tring. Miriam Rothschild makes that clear in the book, as does Ernst Mayr in *The Growth of Biological Thought* (p.272): "The first two authors who clearly described and defined the biological species were the entomologists K. Jordan . . . and Poulton . . .".

What now seems impossible is to

distinguish the intellectual contribution made by Lord Rothschild himself. Karl Jordan said of him in his obituary (*Nature* **140**, 574; 1937) that

he would have gained high distinction in science without a family name already world-famous. His interest was so intense and so wide, his ever-ready support of science so valuable and his scientific publications so important, that he held a high place of honour in zoology and was elected an honorary fellow by many foreign societies. Entomologists, ornithologist, herpetologists and mammalogist all claimed him as one of their own.

Jordan's obituary is far from flattering or conventional; Miriam Rothschild similarly pulls no punches in dealing with Walter or anyone else in the family; but both agree that Walter Rothschild did make a considerable contribution to science. The present book, from its width of scope and aim at a general readership, was not the one in which to analyse the scientific contributions of Tring in detail. (Yet within these limits a very lucid and adequate indication is given.) It would need a considerable expert to go on to such an analysis, but there is one, Miriam Rothschild. □

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Briny tales

Peter J. Smith

The Mediterranean was a Desert: A Voyage of the Glomar Challenger.

By Kenneth J. Hsü.

Princeton University Press: 1983. Pp.197. £15.60, \$17.95.

IN 1961, William Ryan and Brackett Hersey discovered a strong seismic reflector 100–200 metres below the Mediterranean sea floor. There was nothing very remarkable about that, you might think; acoustic reflecting horizons are hardly uncommon in either oceanic or continental crust. But even in those early days of reflection profiling this one looked odd, for it ran parallel to the sea bed, mimicking the highly varied topography above. Moreover, in subsequent years the M-reflector, as it came to be called, was traced throughout much of the Mediterranean. It evidently marked the top of some very extensive subsurface deposit.

Ryan's curiosity on this point was finally satisfied in 1970 when, with Kenneth Hsü, he became co-chief scientist on *Glomar Challenger's* first drilling expedition to the Mediterranean (Leg 13). The M-layer turned out to comprise evaporites (the evaporative residues of sea water), on the surface of which were oozy, basaltic and evaporitic gravels. Though now buried beneath 100 metres or more of marine sediment, which in turn lay beneath 2,000

metres of water, the gravels could only have been the products of *subaerial* erosion. Furthermore, the evaporites themselves contained components (e.g. anhydrite) formed only in arid, shallow-water zones, even though they were underlain by more deep-water sediments.

Ryan's first reaction to the identification of evaporites was to suppose that they were deposited from deep brine pools similar to those in the Red Sea; but the problem with that is that brine pools precipitate calcium sulphate only in hydrated form (gypsum as opposed to anhydrite). Hsü's alternative hypothesis, which originally appeared the more preposterous, was that prior to the five million years of deposition represented by the seabed sediments, the Mediterranean had dried out completely, turning the area into a huge "death valley". This view, backed by several independent lines of evidence, has largely prevailed. For a million years or more the Mediterranean's link with the Atlantic off Gibraltar was evidently broken, allowing the water to evaporate and deposit its dissolved load.

The development of this remarkable scientific story weaves its way through Hsü's text, which would be interesting enough if that were all it contained. But there is much more to it than that, for Hsü also presents a narrative of the complete two-month voyage in 1970, making the book as much a human and social document as it is a tale of scientific discovery. Life in a closed, isolated community has its perils at the best of times; but add millions of dollars worth of equipment any piece of which can malfunction at any time, four distinct groups of people (sailors, drillers, marine technicians and scientists) with different outlooks and aspirations, the clash of scientific egos, the vagaries of the weather, the possibility of illness and the perpetual need to make rapid decisions involving huge amounts of money, and you have a mighty explosion looking for somewhere to happen.

That any project could possibly succeed under such circumstances says much for scientists' determination to overcome both nature's notorious reluctance to reveal its secrets and the inclination of human institutions and artefacts to abet nature in its nefarious purpose. That this book succeeds in imparting the flavour of what it must be like to take part in such a knife-edge enterprise likewise says much about Hsü's skill as a communicator, not only of facts and events but also of hopes, fears, elation, despair and, above all, enthusiasm. Hsü has managed to reach parts of the scientific undertaking that most others have been unable or unwilling to reach; and the result is an impressive, if informal and racy, account, for layperson and scientist alike, of the nerve-racking business of wresting information from the Earth's deeper oceanic crust. □

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Out of this world

Jon Darius

The Atlas of the Solar System.

By Patrick Moore, Garry Hunt, Iain

Nicolson and Peter Cattermole.

Mitchell Beazley/Rand McNally: 1983.

Pp.464. £19.95, \$40.

PUBLICATION of an atlas of the Solar System in 1983 is a shrewd choice. The rich veins of planetary imagery mined by Pioneer, Viking and Voyager probes will not be substantially expanded until Voyager 2 reaches Uranus in 1986. On the other hand, sufficient time has elapsed since the last planetary spectacle to consolidate the observations and map the new territories.

Mitchell Beazley have published a series of four astronomical atlases — *The Moon* and *Jupiter* in 1981, *The Sun* and *Saturn* in 1982 — each pitched at a lay audience and enjoying the cachet of approval by the Royal Astronomical Society. *The Atlas of the Solar System* is a compound of these four publications, somewhat condensed and reorganized, and interlarded with new material on the remaining bodies. The publishers have earned a high reputation for "packaging" their books, and happily the *Atlas* proves the rule: the layout and artwork are exemplary.

Skilful editing generally prevents the seams from showing in the reprinted material, but some quirks occur. Why omit half the photomosaics of the Jovian satellites and half the double spreads on individual lunar regions? Wholesale deletion (or retention) would have been preferable. Let early ring theories, unusual lunar features and the radioheliograph be dropped if need be; but omission of terrestrial aurora looks decidedly odd when both Jovian and Saturnian aurora are included.

The single most serious fault in *The Atlas* lies in its haphazard distribution of material: 30% on Jupiter and Saturn, yet 8% on Mars and a scandalous 3% on the Earth. Asteroids, comets, meteorites and meteors are vouchsafed a scant few pages; zodiacal dust, not a single word. Despite lip service in the preface, the historical dimension gets short shrift. To call the potted biographies of astronomers erratic is too generous.

But the roses indisputably outnumber the brickbats. I know of no single book, popular or professional, with a better compilation of planetary maps (barring the Earth) — legible, up to date and endowed with complete lists of features. The text is clear and informative. If it is not quite the synthesis to which it aspired, *The Atlas* is nevertheless handsome, timely and eminently readable. □

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Historic X-rays and the Big Bang

David W. Hughes

Glimpsing an Invisible Universe:

The Emergence of X-Ray Astronomy.

By Richard F. Hirsh.

Cambridge University Press: 1983.

Pp.186. £20, \$39.50.

The Moment of Creation: Big Bang

Physics from before the First

Millisecond to the Present Universe.

By James S. Trefil.

Charles Scribner's Sons: 1983.

Pp.234. \$15.95.

ASTRONOMICAL frontiers have crumbled dramatically over the past few decades. For example, books on X-ray astronomy and Big Bang physics could not have been written 30 years ago; today they are being produced in profusion.

X-ray astronomy started in the late 1950s, the first X-ray star other than the Sun being detected in 1962. The X-ray universe is one of explosive high temperatures, intense gravitational fields, esoteric concepts such as rotating neutron stars and black holes, and rapid time variations all "visible" only through the aid of sophisticated instrumentation carried above our absorbing atmosphere. Richard F. Hirsh, a scientific historian, has written an account of the growth of X-ray astronomy, the book having blossomed from his dissertation written for the University of Wisconsin.

Hirsh's story makes fascinating reading as we follow the tumultuous growth of this speciality. One paper on X-ray astronomy was published in 1962. Only ten years later the annual rate had risen to 311. And Hirsh found that while there were only four X-ray astronomers in 1962, in 1972 the number was 170. In percentage terms X-ray studies in the field of astronomy leapt from 0.8 to 11.2 in ten years. This is an enormous increase over such a short time and obviously depends on considerable government support and funding, a migration into astronomy of physicists and engineers, and a host of technological innovations. Why did it happen? Hirsh points the finger at the "Pearl Harbor" of the technological war, Sputnik 1.

Once into X-ray astronomy, scientists found the discoveries coming thick and fast. The emissions from the solar corona gave astronomers the first glimpse of the high-energy universe. Some predicted then that non-solar sources would be feeble and undetectable. Others were undaunted, kept an open mind and were rewarded by the detection of Scorpius X 1 on 18 June 1962. The size of the diffuse X-ray flux helped hasten the decline of the Continuous Creation theory.

By 1971 the "glimpsing" phase of X-ray studies characterized by brief rocket obser-

vations was replaced by the survey phase and the Uhuru satellite. Many X-ray sources were seen to be binary systems consisting of a compact object (neutron star or black hole) orbiting large companions with matter transferring between the two. But in the late 1970s the funding for X-ray astronomy decreased. X-ray astronomers lost their favoured position, and became just one of many pressure groups scrambling for money.

Hirsh has written an intriguing book which provides a clear picture of the way a branch of science can evolve — or stumble along — in the United States. I didn't realize that recent history could be so enthralling, and my hope is that Hirsh turns his attention to other astronomical fields and produces more splendid works.

Turning to James Trefil's book on the Big Bang (which started at time $t = 0$), do you believe that the interval between time $t = 500,000$ years and the present "is the least interesting time for scientists"? And do you believe that gauge theories "swept through the community of physicists bringing with them an entirely new way of looking at natural phenomena"? I don't.

And Trefil didn't convince me otherwise, but he made an excellent attempt.

Trefil divides the Big Bang into time intervals — the first 500,000 years, 3 minutes, 1 millisecond and 10^{-35} second. Between 3 minutes and 500,000 years there were just the ions of hydrogen, deuterium and helium plus electrons. At 3 minutes the temperature was 10^9 K and there were six times more protons than neutrons. Earlier than 10^{-3} seconds we are in the hands of the theoreticians.

Why is the isotropic background radiation isotropic? How were galaxies produced? Why is the mass of the universe close to the value needed to stop its expansion? Where has the antimatter gone? Where are the relic particles? Trefil has a gift for tackling such questions for the layman and for explaining extremely complex phenomena. This is a good book and will encourage many to delve deeper into the first 500,000 years. For myself though, I'm going to stick with the present. □

David W. Hughes is a Lecturer in Astronomy and Physics at the University of Sheffield.

Hard particles

Nigel Calder

Quarks: The Stuff of Matter.

By Harald Fritzsch.

Allen Lane/Basic Books: 1983. Pp.300.

£12.95, \$19.

The Cosmic Onion: Quarks and the Nature of the Universe.

By Frank Close.

Heinemann Educational: 1983. Pp.182.

Hbk £12; pbk £5.95.

IN my copy of *War and Peace*, a thoughtful publisher provides a large bookmark on which the names and relationships of Tolstoy's principal characters are set out, together with the patronymics and diminutives. Russian names are confusing. Authors of allegedly "popular" books on scientific subjects often add a glossary at the back, in the belief that this will make a jargon-laden text intelligible. Both books under review have glossaries. In a Russian novel the personal names are embedded in simple phrases having to do with love, anger and everyday human actions. In books and magazines on science, on the other hand, the jargon can accumulate until each sentence is like a motorway pile-up, in which all hope of comprehension lies smashed.

The publishers of *Quarks: The Stuff of Matter* say that it is for the general reader, and written "without recourse to technical jargon". Opening the book at random I came across these sentences: "Quantum chromodynamics, our theory of the strong interaction, is an example of a non-Abelian gauge theory. This theory uses the

relevant gauge group SU(3), under which all colour transformations can be subsumed". The key terms are not even to be found in the glossary. An unfortunate recurrent misprint in this book adds the *myon* and the *myonneutrino* to the already bewildering menagerie of elementary species. Muscular particles, by the sound of them.

The publishers of *The Cosmic Onion: Quarks and the Nature of the Universe* tell us that the author is well known for his ability to popularize the advanced ideas of physics for a general audience, and that this book describes the current ideas "without mathematics". This promise is broken in a mild way by page 16, where we read: $p \times r = h$. Thereafter more and more formulae appear, until by page 111 the text has degenerated into a matrix representation of $W^+ + e^- = \nu^0$.

Two accomplished physicists have undoubtedly laboured hard, and in a restricted sense both have done well. A physics undergraduate who persevered with either book could learn a lot. But then an undergraduate hardly needs cartoon creatures to represent the varieties of quarks, as in Frank Close's book, or jokey poems, as in Harald Fritzsch's. I could not recommend either book to a biologist, never mind a lawyer or a politician.

These books are replete with the wonders of the Eightfold Way, quarks, gluons, charm, the electroweak force, Grand Unification, and the rest. In subject matter there is little to choose between them, and both books refer to the exciting discovery of the W particle announced in January 1983. The general public ought to share the physicists' excitement; cultural considerations apart, Leon Lederman points out

in a foreword to Fritzsche's book that the public has paid the bill for this research.

So what goes wrong? You need an irreducible minimum of terminology to identify the most important particles and the cardinal phenomena; every superfluous technical word beyond that will cost you a hundred readers. It is also a matter of page-by-page comprehensibility. When specialists write for the general reader, they all too often assume that something explained on page 10 can be referred to freely again on page 110. These books require the reader to have almost total recall of unfamiliar concepts.

Then the weird processes of the subatomic world have to be explained as limpidly as possible, to make it clear, for example, that force-carrying particles are matter-antimatter combinations of the kinds of durable particles on which they act. Instead of any such illumination, the reader of the two books under review finds more than he is ever likely to want to know about the decay of orthopositronium or the hyperfine splitting of quark clusters.

Finally, you must jolly the reader along — strew his path with flowers, as Faraday put it. The human adventure of high-energy physics, with its heroic experiments and its races for one Nobel prize after another, provides a ready source of jollification. Frequent reminders that abstruse-seeming processes tell us why the universe is the way it is, and why familiar objects in it behave as they do, can also help. Close works harder at trying to meet this requirement than Fritzsche does.

There ought to be a society for the prevention of mental cruelty to the general reader. (We are all general readers, outside our own areas of expertise.) It took me years to discover that relativity, for example, is quite simple, despite the many "popular" accounts that make it baffling. Readers are too often left feeling stupid but they don't complain because they have no wish to parade what they take to be their own deficiencies, rather than the authors' and publishers'. I doubt whether the publishers of these two books on particle physics, who proclaim their clarity and simplicity, could give any satisfactory explanation of what the books are about. □

Nigel Calder is a science writer. His most recent book on fundamental physics was Einstein's Universe (BBC Publications, 1979).

● We received a third book on elementary particles for the layman after Nigel Calder's review (above) was complete: *The Quest for Quarks* by Brian McCusker (Cambridge University Press; £7.95, \$14.95). Unfortunately, it would appear to fall into the same errors Calder criticizes — but it is relieved by the fact that McCusker (of the University of Sydney) believes he and others have discovered free quarks. Most physicists would reject the claim, but McCusker argues it well in a 32-page chapter. This raises the book above the usual run, and would make it worth reading, say, for half-an-hour in the library. **Robert Walgate**

Art of electronics

W. Graham Richards

Computer Images: State of the Art.

By Joseph Deken.

Thames & Hudson/Stewart, Tabori & Chang, New York: 1983. Pp.200.
Hbk £15, \$25; pbk £9.95, \$16.95.

CLASSICAL painting never recovered from the invention of photography. The fact that a near perfect reproduction of reality can be made has had profound influence on the artist. Now the development of computer image processing has expanded our vision far beyond the range of human senses and is likely to have an even more drastic impact on artists, photographers and film-makers.

Images can be created with which the observer may interact directly. Already it is possible for trainee pilots to "fly" in totally realistic simulated aircraft and for video games to permit battles such as those in the Disney movie *Tron*. The scope seems endless, and many of the contemporary results are quite lovely to behold.

Deken has given an accurate title to his presentation of the state of the art. In no way a technical book, rather it is a collection of some of the finest current examples of computer generated pictures from a wide spectrum of sources and disciplines. It

does serve a scientific as well as a visual appetite. Browsing through the 250-odd highly coloured reproductions gives a clear idea of just what is possible with available technology. Any specialist is likely to encounter some of the pictures from his own publicity calendar, but also to have his imagination stimulated with thoughts of extra things one could achieve with the appropriate facilities.

The author suggests that the combination of computer and graphics display may enhance our vision of the universe in as dramatic a way as the telescope and the microscope. Certainly to anyone working in science at a molecular level this must be true. At last the sterile language of molecular formulae on the printed page can be translated into something which, even if not "reality", is nonetheless a very acceptable and comprehensible metaphor.

The format of a book can, of course, only hint at the extra dimensions available when the images can be made both dynamic and interactive. Despite this necessary limitation, however, the illustrations and accompanying text are sufficient to stimulate both seasoned scientists and graduate students whose careers are likely to be deeply influenced by colour graphics. □

W. Graham Richards is a Lecturer in Physical Chemistry, Oxford University, and a member of the editorial board of the Journal of Molecular Graphics.



"The Second Nuclear Wars Composite", 1982. To create the picture Nancy Burson combined the features of the leaders of the five countries possessing nuclear warheads — Reagan, Brezhnev, Mitterand, Thatcher and Xiaoping. The extent to which each face appears is proportional to the number of warheads in that country.

From Computer Images.

Science for all

William H. Press

The Roving Mind.

By Isaac Asimov.

Prometheus: 1983. Pp.348. \$17.95.

DOGS, and related species, reject excess body heat by panting. Humans generally sweat. Isaac Asimov writes essays. This book is a collection of 62 such essays, whose range of subject matter (and also quality) can be accurately described as "diverse".

At his finest, Asimov is a very good popularizer of science. In an avuncular style that is uniquely his own, he asks questions that his lay reader would like to ask, and then he answers them in straightforward (if blocky and perhaps overly idiosyncratic) prose. There are a number of superb popularizers at work: some are better writers than Asimov (Lewis Thomas or Stephen Jay Gould); some may be more charismatic (Carl Sagan); but Asimov was doing it first, prodigiously and indefatigably. We should not forget, in the current and perhaps faddish explosion of popular science, that there were lean decades during which even the best popularizers wrote "science fact" columns for science fiction magazines. Many who are scientific professionals were once

adolescent readers of those columns.

In this volume, Asimov's best include pieces detailing the present mysteries of solar astronomy, removing the mythology of cloning (backyard gardeners do it all the time), and pointing out deficiencies in the supposed chemistry expertise of Sherlock Holmes.

He puts forward occasional dandy (though debatable) ideas: telepathy must be impossible, he says, because it has such evident survival benefit that animals would

long since have developed it; an all-knowing, computerized government will be less, not more, repressive, he says, since it will selectively repress only a few individuals, not everyone indiscriminately (I do not believe that); collectors of the future will pay high prices for today's expired credit cards; hotel rooms of the future will have, on the outside, featureless doors that respond to your thumbprint, but (he reassures us) on the inside will be the familiar door knob and chain or bolt. We can only marvel at his clarity of vision.

And the rest of the essays? A number might be called political, if that term can be used to include a kind of vague, pro-technical liberalism. Asimov exhorts us all

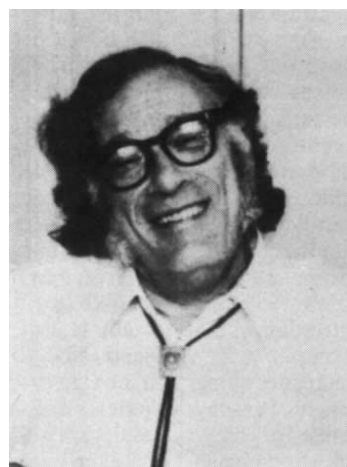
to "control population, conserve energy, increase the humanity, rationality, and wisdom of governments, work for a better ecological balance, and encourage the continued advance of science and technology, using its products more wisely than in the past". He is against creationism, pseudo-science, astrology, censorship, and the (US) "moral majority" movement. These are not entirely uncourageous stands to take in magazines like *Family Weekly* or *T.V. Guide*, where some of the essays

originally appeared.

Technically sophisticated readers may, however, find the rhetoric predictable and even quaint, like some newsletter to an ageing group of party faithful who still get a little thrill at reading the same old arguments that they have believed in for so long.

If Asimov's arguments are still making converts to science and to rational thought, that is for the good. If they are not (and this is my own fear), then

we had better look seriously at why they are failing, at what can be done in popular defence of basic scientific research (as distinct from "high-tech" faddishness) and at who is to do it. □



Asimov — "doing it first . . .".

William H. Press is Chairman of the Department of Astronomy at Harvard University.

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Choice of computers

Donald MacKay

Are Computers Alive? Evolution and New Life Forms.

By Geoff Simons.

Harvester/Birkhäuser: 1983.

Pp.212. £9.95, \$18.95.

A PAPERBACK that caused some excitement in my schooldays was entitled *Meet Yourself as You Really Are*. Similar in plan to a "programmed learning" text, it took you through a labyrinth of questions about yourself, instructing you at each step to turn to a fresh page according to the answer you gave. In the end — hey presto! — it led you to a detailed description of your personality. Despite the sense of awe we felt at such a performance, it did not occur to many of us to think of the book as "alive". Why not? Judged by Mr Simons's logic, we might appear to have been unduly conservative.

Having myself spent a lifetime resisting philosophical arguments that undervalue the powers of computers, I find this book a disappointing mixture of sense and nonsense. Over large tracts of technical territory Simons leads us with a sure foot, a

racy tongue and a perceptive eye. He writes lucidly, and sketches an instructive picture that takes account of most current developments in computer science. Then, recurrently, as with Balaam of old, the prophetic mantle descends and he finds himself constrained to emit oracles of doom against the whole religious view of human nature and human destiny, with the settled conviction that any resistance to his ideas "... is largely a matter of human vanity. Status and self-image are at stake". The old canard is repeated that Copernicus, Darwin and Freud "effectively dethroned man from (respectively) the centre of the universe, a zoological pedestal, and conscious autonomy over all human motivation". "In a number of ways", he claims, "the emergence of computer life is rightly sensed as continuing this process of dethronement . . . It is likely that *there are other species to come on Earth, that their progenitors are already working amongst us, and that human beings may or may not find it possible to co-exist with the new life-forms that will appear*" (pp. 3-4, italics original).

No doubt some case can be made for redefining the concept of "life" to cover computers — yea doubtless even programmed-instruction books, which can be regarded as computers with do-it-

yourself lookup. The question (as with some contemporary redefinitions of "democracy"), is how many of the old associations of the term would then become misleading or downright deceptive. Simons is right to stress the greater importance now attached to information-processing in framing concepts of life; but his more exotic argumentation repeatedly founders (like the classical "Turing test") on a confusion between *necessary* and *sufficient* conditions. He seeks support from the usage of evolutionary biology in terms which (if only he had his tongue in his cheek) could be read as a salutary take-off: Computers "feed on electrical energy and excrete heat energy" (p.15). "Assembly, either of hydrocarbon molecules (for plant and animal life) or of nuts and bolts (for machine life), may be represented as a common reproductive mechanism" (p.19). Computers, like biological species, "evolve". "The growing intimacy between successive computer generations suggests such concepts as kinship, the 'handing down' of proven adaptive features, and resemblance between parent and progeny. Considerations of this sort suggest that it might be useful to classify, in biological terms, many of the emerging computer systems" (p.26). The role of human beings in "computer reproduction" is compared with that of humble bees in the reproduction of clover (p.19).

Confusion of categories bedevils the discussion of implications for our view of human nature. Because in a computer program "jumps can be *conditional* (in other words [*sic*] the computer can *decide* . . . whether the jump should be made)", either "computers have free will, or . . . humans do not" (pp.148, 149). What Simons fails to see is that this argument proves either too little or too much. The human nervous system itself embodies many "conditional jumps" in its programs (for example in the control of locomotion) at levels where nobody in his senses would claim that there is anyone making "free choices". It is people — conscious agents — who make choices, freely or otherwise. Even when they do so, to describe their *brains* as "choosing" would be a mere misuse of language — a category mistake. To turn every conditional jump in a computer into an act of free will would be to attribute to every contact-controlled traffic light something it would not make sense to attribute to a human brain. The meaningful and interesting question is what *kind* of conditional-jump structure in the human CNS embodies the process we know at first hand as conscious choosing, as distinct from all the others that do not. To the clarification of such issues Simons's terminological euphoria makes only a negative contribution. □

Donald MacKay is Emeritus Professor of Communication and Neuroscience at the University of Keele.

Dreams and schemes in the dark

David Cohen

Landscapes of the Night: How and Why We Dream.

By Christopher Evans, edited and completed by Peter Evans.

Gollancz: 1983. Pp.254. £7.95.

In 1964, Christopher Evans, the author of *The Mighty Micro*, published a paper in which he drew an analogy between the state of dreaming and a computer being off-line. In both cases, contact with the real environment was cut. This new book, *Landscapes of the Night*, is an extension of that 1964 paper. Evans died suddenly in 1979 and the task of editing and completing the book was taken on by the science journalist, Peter Evans, who had the problem of thinking the early drafts through to a logical end. It is perhaps not surprising that a book written in this way, by two minds with little contact, should fall short of its rather grand aims.

Christopher Evans clearly wanted to state a brand new theory of dreams. The book begins, however, with a potted introduction to the physiology of sleep and dreams, to the ideas of Freud and Jung and, less conventionally, to the relationship between dreams and extra-sensory perception. It then moves on to describe research in the 1950s in which aspirants to an entry in the Guinness Book of Records tried to stay awake for 200 hours or more. All of them began to hallucinate, became very ill-tempered and, eventually, had to be allowed to fall asleep. Since then, more sophisticated research has shown that we dream when we have rapid eye movements and that if subjects are deprived of REM sleep, they also become psychologically disturbed. Evans concludes that we need to dream. If so, why?

Freud believed, of course, that he had the answer. But Evans dismisses Freud's ideas as mere wishful thinking, and begins

his explanation of why we dream by looking at the work of the French neuro-physiologist, Michel Jouvet. Jouvet made lesions in the brain stem of cats which removed the muscular inhibition that prevents movement while dreaming. The cats could then "act out" their dreams. Jouvet reported a frenzy of stereotyped aggression in which cats savaged mice and, from time to time, cowered from fierce dogs. Jouvet concluded that REM sleep allows the animal to rehearse or "see" its basic instinctual repertoire.

Evans here made a logical leap. Cats need to kill mice to survive. But human beings are more subtle and need social skills to survive. Harking back to his computer analogy, Evans argued that, in dreaming, all the programs that we need to function are being updated. Tomorrow, you may need to be nice to the boss and may make a pass at that attractive biochemist. Better run through the game plan in the dream box during the night! That is why babies need to dream more; they have more to remember and learn.

It all sounds pat but is philosophically rather dim. Just who is sitting in the cortex during the night, getting the benefit of these reels and reels of clever social planning? Is it Ryle's Ghost in the Machine who has come back to get a crash course in etiquette and social skills? And how is the ghost to pass all the information on to me who actually has to make the pass at the biochemist? Social life has conscious and instinctive aspects but our plans and reflections surely need conscious attention to be of any use. The book does not see this as a problem. It prefers the neat answer — dreams help you cope.

The book also ignores day-dreaming and the fact that we often consciously imagine how we will behave in situations. Is this like dreaming or is it something utterly different?

As a popular book, *Landscapes of the Night* deserves to do well, being both informative and entertaining. But as a serious contribution to dream work it is rather slight. □

David Cohen is the editor of *Psychology News*.

The Biology of Women

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Darwin in luxurious surroundings

J.S. Jones

Charles Darwin: A Centennial Commemorative.

Edited by Roger G. Chapman and Cleveland T. Duval.

Nova Pacifica, Wellington, New Zealand/Croom Helm: 1982. £350, edition limited to 750 copies.

CHARLES Darwin received an annual income of £350 from the *Origin of Species*. By spending only one year's royalties from his greatest work he could now afford to buy this extravagant celebration of its contents. Entirely produced in New Zealand, and bound in calf by the Government Printing Office, 750 copies have been printed to grace the shelves of discriminating Darwinians. Eleven chapters by different authors discuss Darwin's life, the *Beagle* voyage, and the impact of Darwin's work on science, religion and society in the nineteenth and twentieth centuries. The book's contents are of as much interest to the historian as to the biologist, and the diversity of topics and authors leads to a certain unevenness. Thus, in one chapter we are told that DNA can be seen (if suitably stained) to make up threadlike structures called chromosomes, while elsewhere we learn that Stradivarius violins and circuses are among the products of evolution which probably do not result directly from natural selection.

The quotations from Darwin and his contemporaries contrast in their vigour and directness with the pallid academic prose which too often surrounds them. Responses to the theory of evolution were extreme: among geologists, Geikie saw the *Origin* as a "great symphony . . . where the deep undertones of geology seldom fail to be audible", but Sedgwick "laughed at parts of it until my sides were almost sore".

Social theorists had equally opposed and eloquently expressed views. To many, evolutionary theory justified the existing order. T.H. Huxley claimed that Darwinism should prove to the working man that "it is better for himself, better for his own people, and better for future generations that he should starve than steal". Walter Bagehot, however, said of Adam Smith's attempts to trace the evolution of human societies that they showed "how, from being a savage, man rose to be a Scotchman".

Quotations from the great Victorians are accompanied by many contemporary illustrations, including no less than six cartoons showing Darwin as an ape. Some of them seem to have been chosen mainly for decoration, however; for example, a gloomy lithograph of a railway accident is included because of the effect which the contemplation of such disasters had on the religious views of Herbert Spencer.

There are also some unexpected insights into Darwin himself. His most abiding memory of his voyage around the world was of "one continual puke", and in a fit of absent-mindedness in South America Darwin cooked and ate the first specimen of the flightless bird *Rhea darwini*. We discover also that although in his first year at Edinburgh Darwin took out 46 books from the University Library (including Pemberton on Viscera), in his second year he took out none at all. It is rather a surprise to find how close Darwin came to the Mendelian ideas of dominance and recessivity; his plant breeding experiments showed that the characters of the offspring depended on "the same character being present and visible in one of the breeds which are crossed, and latent or invisible in the other".

All in all, this book is a worthy attempt to put Darwinism into its historical context. However, most students will have to wait for the paperback. □

J.S. Jones is in the Department of Genetics and Biometry, University College London.

Look at primates!

R.D. Martin

A Complete Guide to Monkeys, Apes and Other Primates.

By Michael Kavanagh.

Cape/University of Oregon Press: 1983. Pp.224. £10.95, \$19.95.

BOOKS on primates, at any level, have a wide potential readership and the demand has been met by a constant flow of new publications. Confronted with such a wide choice, it is difficult for the general reader to select a popular book that reflects (as it should) a distillation of established knowledge. With Michael Kavanagh's *Guide to Monkeys, Apes and Other*

Primates, such readers can rest assured; Kavanagh has certainly done his homework to produce a text that is basically reliable as well as being eminently readable.

The words "complete guide" in the title are no idle boast. There is a substantial account of every genus of living primate and each genus profile is accompanied by an accurate distribution map and at least one colour photograph. Each profile begins by providing useful standard data (e.g. on physical characteristics and body weight), but then concentrates on summarizing present knowledge about the natural behaviour and ecology of primates. Here lies the book's greatest strength: Kavanagh has clearly made a concerted effort to familiarize himself with the vast literature generated by several decades of detailed primate field studies. The excellent

series of genus profiles is supported by a perceptive introductory discussion of basic primate characteristics and by a useful appendix that covers the details of primate classification (thus freeing the main text from cumbersome detail).

Most of the photographs were taken under natural conditions, though for certain poorly-known species it was obviously necessary to fall back on pictures of captive animals. The choice of photographs underlines the concern with the natural habits of primates and also reflects the theme of conservation, which is prominent throughout the text. Of the 183 primate species appearing in the appendix, 67 (more than a third) are cited as being listed in the *Mammal Red Data Book* as threatened with extinction — the wild



From *A Complete Guide to Monkeys*, . . .

A slow loris, "creature of the night".

populations of several species or subspecies have now been reduced to the hundreds, including those of the aye-aye, the golden lion tamarin, the woolly spider monkey and the mountain gorilla. The plight of these particular species is specifically covered in the text and at the end of the book there is a general discussion of problems in primate conservation.

Considering the wide coverage of this book and the aim of presenting information in simple, readable form, there are remarkably few errors. One example is provided by an unfortunate reference to the primate fetus as "nourished by a particularly intimate mingling of its blood system with that of its mother", which ignores the non-invasive epitheliochorial placentation of lemurs and lorises. It is also a pity to see certain standard myths repeated, such as the suggestion that the primate snout shortened because of reduction in olfactory powers (forgetting the fate of teeth attached to the underside of the snout) and the reference to the aye-aye's "enormously long and thin middle finger" (whereas, as in all lemurs, the fourth digit of the hand is the longest). But these are small flaws in a very competent book. Anyone seeking a rapid and useful introduction to the behaviour and ecology of primates need look no further. □

R.D. Martin is Professor of Physical Anthropology at University College London.

World of ornithology

John H. Andrews

COUNTLESS birders in North America and Europe took their first steps in bird identification with a "Peterson guide". When *A Field Guide to the Birds of Britain and Europe* (Collins, £7.95) was first published in 1954, Roger Tory Peterson's illustrations were a revelation: overnight, bird identification became easier and it is arguable that this one artist has contributed more than almost anyone to the popularization of birdwatching.

Soon, other guides came along but, four revisions and seventeen reprints later, this one is still in business. The latest edition prints in colour several plates formerly black-and-white and adds new material. Unfortunately, some of the reproduction is not up to the original standard, while identification skills have progressed so far that Peterson might, for some of the trickier species, now emphasize different distinguishing features from those he selected originally. However, Guy Mountford's updated text deals adequately with these and Phil Hollom's distribution maps have also been revised. For this reason, owners of earlier editions may find this one worth acquiring: it certainly remains a good and serviceable guide for any newcomer.

The massive strides made in field identification of birds over the past 30 years are exemplified by *Seabirds: An Identification Guide* (Croom Helm, £15.95). Here, after 11 years of field work, Peter Harrison has brought together current information on the appearance and ranges of all the world's seabirds, including species such as grebes and phalaropes. Even with these, the total is only just over 300 — a reflection of the limited niches available to birds in marine habitats. However, their small number does not make seabirds a simple group to identify. Plumage variations are considerable, especially in species such as albatrosses which take up to seven years to mature. The author has sought to depict all typical forms, with an average of five colour illustrations per species, while his text stresses helpful points of appearance and behaviour. Many distribution maps are tentative but the guide's publication will materially aid the accumulation of further knowledge of range and movements, hitherto beset by the problems of identifying a group of birds mostly "seen from an unstable deck or wind torn headland, as often as not disappearing behind a moderate swell, reappearing fleetingly and at an increasing range".

Apart from the skill required, seawatching needs patience and cold tolerance, so it's small surprise that other species groups have more popular appeal. Birds of prey must be high on the list of most-watched birds, which is just as well given the range of threats they now face. In

Birds of Prey of Southern Africa (Croom Helm, £28.50) Peter Steyn summarizes the present distribution, life histories and status of the 80 species of diurnal raptors, vultures and owls which occur below 15° South, and provides a guide to their identification.

The book includes many useful black-and-white photographs, plus excellent identification plates by Graeme Arnott. The text is information packed but thoroughly readable. It is also an important assessment of current raptor conservation problems, which must be expected to spread as human pressures intensify throughout Africa.

Owls, too, are an extremely popular subject group, especially with publishers who apparently feel that their human facial characteristics give them high marketability. It sometimes seems that they are more written about than studied, and Tony Wardhaugh's *Owls of Britain and Europe* (Blandford, £7.95) is another regurgitation of familiar material, though quite suitable for anyone who has not yet bought his first owl book. By contrast, Heimo Mikkola has made a real addition to ornithological literature with his *Owls of Europe* (Poyser*, £16.80). A comprehensive review of current knowledge, the book opens with general consideration of owls' origins, taxonomy and anatomy. The bulk of the text comprises extensive discussion of the 13 European breeding species, plus four which occur in North Africa and the Middle East, while the final section considers ecological relationships — sexual dimorphism, diet, interspecific aggression, prey populations and isolation mechanisms are examined, and the author is cautious in his conclusions. The work is well supported by bibliography and index, by a range of informative photographs, copious maps and figures, and with meticulous colour plates prepared by Ian Willis.

Bird-booking is almost as diverse a hobby as birdwatching itself and for many the mental stimulus of a Heimo Mikkola will be matched by the idle pleasure of browsing through a book like *North American Marsh Birds* (Harper & Row, \$44.95). Gary Low's big plates, mostly in colour, are indeed handsome and avoid being too photographic. The fact is that the eye doesn't see birds as they are — it picks out the coot's huge feet or the angularity of a gull's beak and somehow emphasizes them in mind and memory. Nor does real light portray birds' plumage as it is — especially in wetlands, reflections make birds change shade and even shape in the blink of an eye. It is pleasant to see marsh birds as they really do look, rather than as slide rule and studio lamp would have it. A disarmingly relaxed narrative text by

*T. & A. D. Poyser, Town Head House, Calton, Waterhouses, Staffs ST10 3JQ, UK. Distributed in North America by Buteo Books, PO Box 481, Vermillion, SD 57069, USA.

... world of ornithology

William Mansell complements the pictures, informing as it entertains.

For the compulsive bird-booker, **The Birdwatcher's Companion** (Robert Hale, £15.95) offers almost limitless browsing. Subtitled *An Encyclopedic Handbook of North American Birdlife*, it is an unusual blend of fact and opinion, of fundamental bird biology and ornithological trivia. The 1,250 entries are arranged alphabetically and it is hard to find anything of significance that is not included. Though centred on North American birds — so that it includes descriptions of North American birding "hot spots", of all occurrences of vagrants and so on — there is much of general interest and the book is at least a partial substitute for Landsborough Thompson's *New Dictionary of Birds* (Nelson, 1964), now hard to obtain and outdated in some respects. Nor is Chris Leahy's text any the worse for being written with style and humour. Even egg



From North American Marsh Birds.

Marsh bird — a yellow-crowned night-heron, depicted by Gary Low.

collectors, whose activities are dismissed as appealing "only to a kinky few" will presumably enjoy the list of vernacular birds' names included "because they made me laugh". Birding is pleasurable after all.

The Birdwatcher's Activity Book (Stackpole, \$11.95), is designed to get browsers out of their chairs and into the field. Starting with the basics of birding skills, the author gently leads the nervous, non-scientific reader to realize that many of his identification judgements depend on knowledge about where birds live and how they behave; he thus already knows something about ecological isolation, in which case why not look at it a bit more closely? The bulk of the text is project-related, with special emphasis on waterfowl, birds of prey and town birds — perhaps the most popular or accessible groups. Because it is based on North American species, the book wouldn't much help birders elsewhere, which suggests a niche for other authors to fill.

As birdwatchers develop confidence so they travel further afield, but inevitably

there are few popular works on the birds of countries where birding is not an indigenous hobby. Majorca and the other Balearic Islands have enjoyed the attentions of many visiting ornithologists and a few residents, but lacked their own definitive work until now, with the publication of **The Birds of the Balearics** (Croom Helm, £29.50). This is, sadly, the last work of David Bannerman who died before he had completed the text: fortunately, his wife Mary was able to finish the work from their joint records. Donald Watson provides some pleasant illustrations, but the book must be used together with a field guide, its main value being the information on status and distribution of species in this island group.

On islands in particular, one becomes very conscious of the influence of weather on bird movements. Indeed, many birders travel to islands specifically to exploit the goodies which, carried off their migration routes by changing weather conditions, make landfall outside their normal ranges. It is a slightly bizarre business that twitchers take particular pleasure in these lost vagrants, each small personal avian disaster a gratifying tick on one's year list. However, that aside, weather is a most important influence on birds. Yet little has been written on the subject, so that **Weather and Bird Behaviour** (Poyser, £12.60) provides a very welcome review of the meteorological aspects of the avian environment. A meteorologist pursuing ornithology as a hobby, Norman Elkins opens the book with an explanation of how weather works and of the weather systems affecting the British Isles. Turning to the birds' response, chapters cover flight, feeding, breeding and comfort — the responses to low temperature, precipitation and wind. Four chapters are devoted to migration — its inception and progress; the phenomenon of drift and displacement; vagrancy; and the migration of soaring birds. The effects of extreme conditions are specifically discussed, as are seabirds, whose response to weather is rather different from that of land species. The text must have widespread application since, though the pattern of weather behaviour varies from place to place, its general principles and the birds' response are similar. And it is pleasant to find that, in Elkins's view, the lost vagrant is not just a wasted life: "Arguably one of the more exciting events of a birdwatchers' career is the discovery of an individual bird which is clearly thousands of kilometers from the edge of its range or migration route . . . an analysis of the details of all the records, can result in important inferences. What appear to be random occurrences may fall into a pattern which gives us a clue to the movements or population dynamics of a particular species". I stand corrected! □

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Paper snakes

Colin McCarthy

Snakes of Australia, 2nd Edn.

By Graeme F. Gow.

Angus & Robertson: 1983. Pp. 118.

Pbk £6.95.

AUSTRALIANS have, over the years, been well-served by guides to their snakes. Since Krefft's *Snakes of Australia* (Australian Museum, 1869) a number of hand-books have been produced; one of the latest upholders of this important tradition is Graeme Gow. In Krefft's day only 67 types of Australian land snakes had been recorded, but now over double that number are known.

Most of Gow's book is devoted to accounts of the range, identifying features and habits of individual species. Other topics considered include a general review of habits and the care of snakes in captivity together with recommendations for the treatment of snake bite. In his introduction, Gow indicates that the aim of his book is "to assist the layman with the identification of all known species"; so it is a pity that, with the exception of one to the Blind snakes, no identification keys are provided. In this respect H.W. Cogger's *Reptiles and Amphibians of Australia*, published by Reed in 1979, is a more helpful work. However Gow's new paperback is a cheaper alternative for those whose interest in Australian herpetology does not extend beyond snakes, and it is also of a more convenient size for field use.

Owners of an early (1976 or 1980) edition are advised that the recommendation, formerly given, that a tourniquet be applied immediately in the event of snake bite, has "now become outdated"; instead immediate application of a broad constrictive bandage together with immobilization of the affected limb in a splint or sling, until medical aid is given, is suggested. This most recent edition is also quite extensively revised in other respects, particularly with regard to nomenclature and species accounts; five new colour plates have been added. Although there is a claim, on the back cover, that it is a "field guide to all Australian land snakes", there are some omissions. Readers may, for instance, be bewildered by Gow's statement (p.48) that the water snake *Enhydrys macleayi* "should produce similar litters to *Enhydrys polylepis*"; *E. polylepis* has not been included in this edition.

In spite of such minor reservations this book is undoubtedly a valuable addition to the herpetological literature; a work that anyone with an interest in snakes would be pleased to own, especially for its 48 pages of mainly very good colour photographs. □

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Herpetology after Smith

Brian Groombridge

Reptiles and Amphibians in Britain.

By D. Frazer.

Collins: 1983. Pp.256. £11.

IN the field of herpetological literature, whether academic or popular in tone, Malcolm Smith is a hard act to follow. Deryk Frazer's book, the latest (number 69) in the valued *New Naturalist* series, is an attempt to replace Smith's earlier and much revised book (number 20, *The British Reptiles and Amphibians*) in the same series. It was decided that the old book would not bear further revision to the extent demanded by recent research. It is pertinent to compare the two works.

The new may indeed replace the old, but in this reviewer's opinion, it does not supersede it. Smith's book, first published in 1951, was written during his retirement to Britain after a quarter of a century spent in the tropical Far East. Smith was a worker of great international repute, with immense field and museum experience. His book on the British herpetofauna fully reflects this experience; his masterly assembly of information, his liberal use of obscure but relevant quotations, and his elegant prose, are redolent with an air of gentlemanly scholarship, reflecting a tradition now sadly past.

Unfortunately, the volume of information now available on amphibians

and reptiles in Britain is great enough to almost prohibit making a similarly coherent and all-embracing exposition today. Deryk Frazer has made a valiant attempt at this task; however, whilst many new research data are incorporated, his text often epitomizes the case of failing to see the wood for the trees.

The general organization of the old book and the new are broadly similar, with accounts of each species being preceded by an overall introduction to the larger group to which they belong. In each instance Smith's book succeeds in providing an effective overall view that Frazer's book does not quite achieve, no matter how many new or additional data are incorporated. Identification and anatomical data are abbreviated and inadequately illustrated. However, Frazer does include a valuable chapter on conservation. Another shortcoming of Frazer's book is the clear bias towards amphibians. Whilst this reflects the author's chief area of expertise, it is disturbing to find no mention of some relevant literature. Despite such drawbacks, Frazer's book certainly provides a convenient review of much recent research on the British herpetofauna, a good lead into much of the relevant literature, and will tell the enquiring naturalist much of what he or she wants to know. All herpetologists and most naturalists will need and wish to read it. □

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that is illustrated. There is also a chapter on how to set about collecting fossils, and advice on proper attitudes towards the non-renewable resources of our fossil beds is given.

David Lambert's *Collins Guide to Dinosaurs* (in the USA, *A Field Guide to Dinosaurs*) is surely the most complete dinosaur book ever published. It lists, and describes briefly, nearly every dinosaur genus ever described. This portion of the book is very up to date, including many of the genera that have been described in the last few years, often in relatively obscure Russian, Chinese and South American journals. There are even some as yet unnamed dinosaurs that are described here on the basis of press reports and personal communications. The other particularly useful sections include a series of maps of all known dinosaur localities (including details of those in Mongolia and China) and a long list of dinosaur museums of the world, each with a summary of the most important exhibits. This book is a delight for any dinosaur fan (like myself) since it is the best quick source for the vital statistics on all known dinosaurs. If only the author could publish a bibliography of all the works that he must have consulted!

The text is pitched at late teens and adults, but younger dinosaur fanatics will doubtless pore over it excitedly and make sense of it by use of their overdeveloped command of polysyllabic words. The quality of production and the drawings are very fine. However, unlike Fortey's book, there is not enough room in *Collins Guide to Dinosaurs* to give an extended discussion of what all of these genera of dinosaurs mean. There are short sections on the biology of each group and on evolution, feeding, physiology, extinction, and so on, but other currently available books have more discussion of these matters.

I feel that Lambert has been constrained by the organization of the book into giving less information than he could have done. Many of the genera included here for the sake of completeness are really indeterminate — single broken teeth and the like. Further, every genus is illustrated by a silhouette pictogram to show size and shape, and each is plotted on a general locality map at the foot of the page. This, unfortunately, means that only a few of the better-known genera have been illustrated as actual fossil bones or as reconstructions. Nevertheless, I feel the book will be a best-seller because it is the most exhaustive dinosaur book ever published — it is literally bulging with more names of dinosaurs than have ever been seen together in one place before. It will be enough to keep those interested in dinosaurs quiet for a very long time while they contemplate such mouthfuls as *Therizinosaurus*, *Szechuanosaurus* and *Muttaborrasaurus*. □

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Past glories

Michael J. Benton

Fossils: the Key to the Past.

By Richard Fortey.

British Museum (Natural History):

1983. Pp.172. Pbk £6.50

(£5.95 at the Museum).

Collins Guide to Dinosaurs.

By David Lambert.

Collins/Avon: 1983. Pp.256.

£6.95, \$8.95.

MANY fine popular books about palaeontology have been published recently. It is a pleasure to review two new books that provide different approaches to the subject and do so in an attractive and authoritative way.

Richard Fortey has done two jobs in his book *Fossils: the Key to the Past*. Firstly, he has presented an interesting account of the uses and relevance of palaeontology in general, and dispels the outmoded stereotype of palaeontologists as old fossils themselves. Fortey shows well the present emphasis in palaeontology on the study of past environments, functional mor-

phology, evolution, extinction and stratigraphy. There are especially good sections on "bringing fossils back to life", on extinction (with a very level-headed discussion of the extinction of the dinosaurs in which the author concentrates on what we do know rather than what is speculation), on the early life of the Precambrian and Cambrian, and on the uses of microfossils in stratigraphy.

The second task that Fortey has performed in his book is to inspire and instruct the amateur fossil collector. Many people are fascinated by the beauty of fossils and they build up collections often with a great deal of effort and expense. Several coffee-table books containing sumptuous illustrations of fine fossil specimens have been published recently, but the supporting text has often been indifferent. A large part of the present book is illustrated by excellent photographs, many in colour, and these will certainly attract many purchasers. The only poor photograph is one taken through the fossilized lens of a trilobite eye (p.86)! The specimens illustrated are all very fine, and yet most of them are not too difficult for the amateur to collect. Fortey has appended notes on the occurrence, significance and preparation of each specimen

The writing on the wall

Glyn Daniel

The Stars and the Stones: Ancient Art and Astronomy in Ireland.

By Martin Brennan.

Thames & Hudson: 1983. Pp.208.

£12, \$19.95.

IN 1740 the Reverend William Stukeley in his *Stonehenge, a Temple Restored to the British Druids* noted the fact that the axis of Stonehenge and the avenue leading from it were directed to the north-east "whereabouts the sun rises when the days are longest". From Stukeley's time onwards there has always been an interest in the astronomical significance of prehistoric megalithic monuments, and this interest in archaeoastronomy (or astro-archaeology) has recently been revived by the writings and speculations of Hawkins and Thom. Its history has been well set out by John Michell in his *A Little History of Astroarchaeology: Stages in the Transformation of a Heresy* (Thames & Hudson, 1977) where he very properly describes Sir J. Norman Lockyer, for fifty years editor of *Nature*, as the founder of this scientific discipline.

The author of this book was born of Irish parents in New York, took a degree in Visual Communication, travelled to Mexico where he did research on prehistoric art, and then to Japan where Kimotaro Kitamura urged him to go to Ireland and study ancient Irish culture. This he did and has lived in Ireland for many years making a special study of the orientation of the Irish Passage Graves, and of their art. His first book, *The Boyne Valley Vision*, published, with a curious appropriateness, by the Dolmen Press in 1980, attracted a great deal of attention

because he claimed the Passage Graves were "timepieces, cosmic clocks", that "the Boyne valley inscriptions represent the earliest form of written communication", and that "geometry did not begin on the banks of the Nile: it began on the banks of the Boyne".

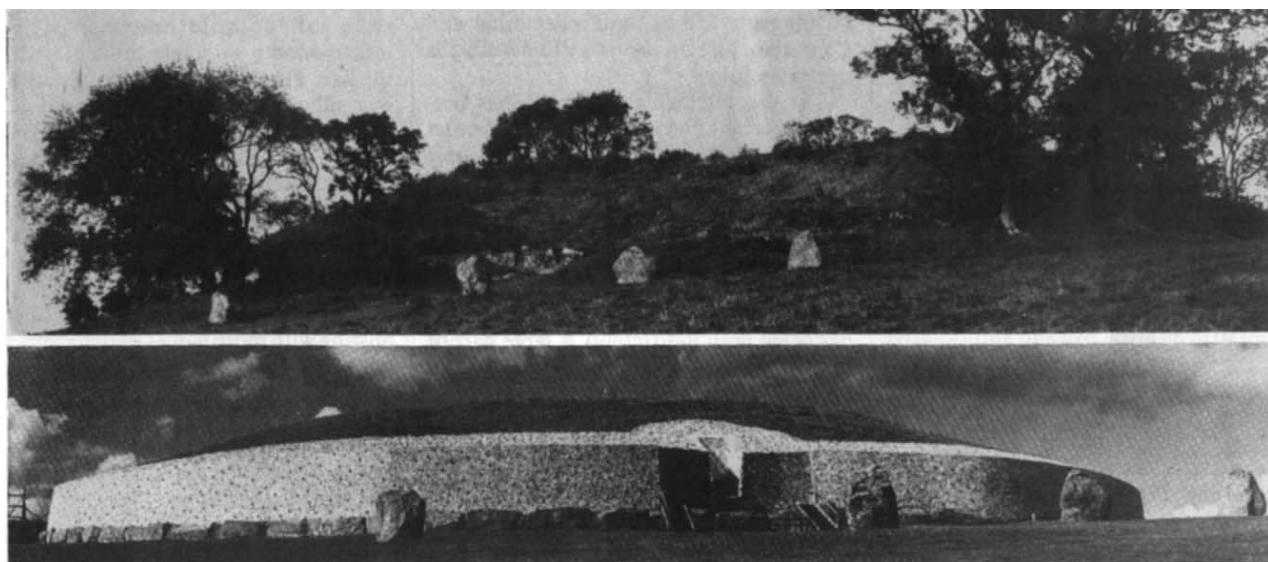
Now, three years later, he develops these apparently extravagant ideas with a wealth of diagrams and illustrations. No one ever doubted that Stonehenge was orientated to the Midsummer sunrise. Many were fascinated when it was discovered that Newgrange was aligned on the winter solstice, and now Brennan appears to have shown that many of the Irish Passage Graves have significant orientations and are, as he puts it, "synchronized to the beacons of the sun". Brennan declares megalithic studies to be "a war of ideas" and I subscribe to this view; but I would not agree with him when he says that the theodolites of researchers such as Thom and himself "clearly reveal the remains of a people deeply concerned with measuring time". Thom, Brennan and other archaeoastronomers turn the orientation of megaliths into part of exact and carefully planned observatories. Aubrey Burl is surely right when he says that the orientation of megaliths is symbolic, ritual and general — not specific and exact. Christian churches are orientated to the east, and mosques to Mecca, but this does not make the temples of Christians and Moslems into observatories or cosmic clocks.

As he develops his thesis, Brennan becomes more involved with what is usually called megalithic art, but for which Sir William Wilde, famous father of his famous son Oscar, coined the delicious word "tymboglyphics" or tomb writing. Many have tried to read this alleged writing, some declaring it to be Phoenician or Egyptian. Most of the specialists in megaliths who have paid attention to the scribings on the walls of the chambered structures, while noting signs that look like

rayed suns and serpents and observing the appearance of spirals and oculi-designs, have felt that the totality of the designs was undecipherable, and that we would never understand their meaning. Brennan does not agree: he thinks the art is directly related to the concept of the Passage Graves as "accurate sun chronometers whose structures are a celebration of light and measurement, and that there is a clear and distinct link between megalithic art and the astronomical events that animate megalithic structures giving them meaning and function".

We all invent the past, and there are no necessary reasons why the informed guesses and theories of established archaeologists and historians should always be right, and the uninformed guesses and theories of amateurs like Hawkins, Thom and Brennan should always be wrong. The general public will find this book interesting and full of food for thought, though some of the diagrams (like that of Barclodiad y Gawres, p.195) seem to introduce details unfamiliar to myself and the late Professor T.G.E. Powell who excavated the site and recorded the scribings with care. And they will chafe at an index that does not include references to authors and sources quoted. It is the credibility of the guesses and theories that we must all, independently, assess. In my own assessment, Brennan's claims that the Passage Graves display "numerous variations of the same sky-imagery which relates astronomical observations to cosmic models of rotating spheres and shells and the criss-cross patterns they generate", and that the art "reinforces the communicative power of the symbols to the extent that they become universal and timeless imprints of mankind's fascination with the sun, the moon and the stars", are not cogent, convincing or credible. □

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Barrow by the Boyne — Newgrange (top) before and (bottom) after reconstruction.

From *The Stars and the Stones*.

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The products featured this week range from a micromanipulator for transferring embryos to a kit for detecting PCBs

● Sono-Tek has released a new line of products for its electronically-operated ultrasonic nozzle systems. The 8308 series of integrated nozzle/pump systems consists of a corrosion-resistant ultrasonic liquid atomizing nozzle and metering pump, together with a control cabinet that houses nozzle controls and flow rate adjustment controls. The Sono-Tek ultrasonic nozzles are non-cloggable. The atomization process itself does not depend on pressurized liquid or air. The rate of delivery of liquid to the spray head can be adjusted from zero flow to the nozzle's maximum capacity using a front panel control knob.

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● The electronic Calibrator type EC is a new class 0.1 instrument from Thommen. The instrument consists of two basic units: the pressure simulator and a newly-developed electronic module. The measurement range is -300 to 1,500 mbar. Pressures up to 3,000 mbar outside this range will not cause damage.

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● The Clor-n-Oil polychlorinated biphenyl (PCB) screening kit from Dexsil Chemicals detects the presence of PCBs in dielectric fluids. The kit can screen samples above and below 50 p.p.m. PCB. It consists of reagents packaged in glass ampules that are broken selectively. The final colour of the solution indicates the presence or the absence of PCBs.

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● Welded steel wire racks that are epoxy-coated are available from Bel-Art Products. These Poxgrid slant racks have angled side bars that give a 5° slant for aerobic culture tubes and a 20° slant for anaerobic culture tubes. The racks can also be used in an upright position.

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● A mechanical shock and vibration sensor, the PKS1-4A1, has been added to the Murata Erie range of ceramic element sensors. Maximum sensitivity lies in the frequency range 1-3 kHz but the sensor will deliver a minimum of 40 mV G⁻¹ over the frequency range 10 Hz to 1 kHz. The operating temperature range is -20 to 60°C and output capacitance is 10,000 pF measured with 1 kHz input at 25°C.

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● A computerized platelet aggregation system is now available from Payton. Using an Apple II microcomputer, Payton has produced a system which can be linked to any aggregation modules. The software package allows inducer concentrations to be automatically computed for final concentrations, and provides audible and visual prompting for all necessary user tasks. For later retrieval, reproduction or analysis all data are stored on a diskette. Patients' results are provided in hard copy (A4 format) by means of any suitable 80-column graphics printer. The system is distributed in the UK by Centronic Sales.

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● Avok has introduced a high-pressure valve actuator. This electric actuator can be used with valves such as the Rheodyne 7000 series without the use of pneumatics. Facilities built into the actuator include the remote indication of position and easy interfacing with computers and controllers. The actuator may be stacked for valve switching applications.

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● Instrument Technology is now offering its TF 1850 fast photodiode with a magnesium fluoride input window. This lowers the instrument's sensitivity to 110 nm. The diode is supplied in a 50-Ω impedance-matched mount and operates from a 3-kV negative supply. It is available with a choice of S20 or solar blind photocathodes. The detector has a 100-ps risetime so that it can be used for examining the pulse shape of excimer lasers.

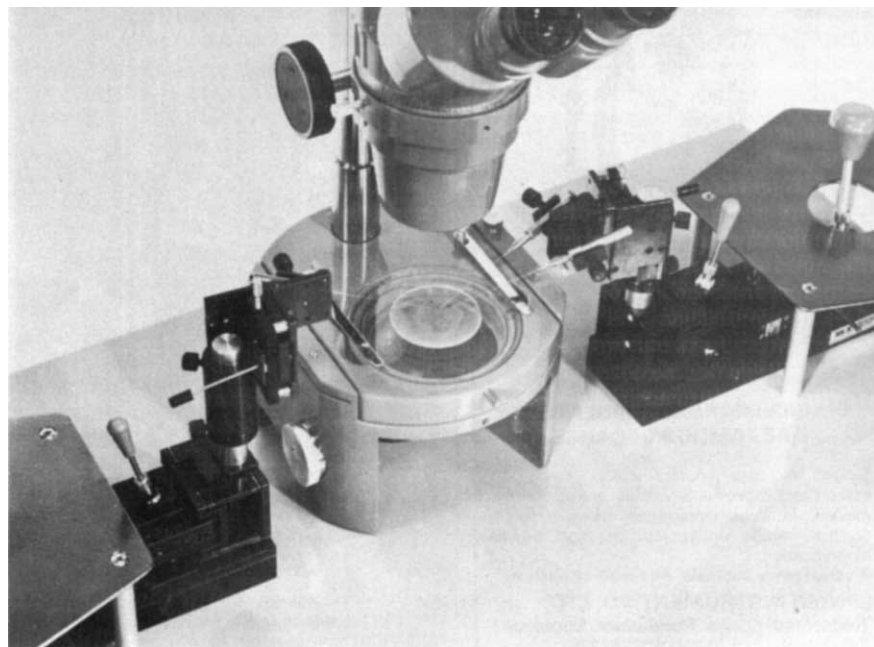
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● Research Instruments has introduced the TLO125 micromanipulator for executing the techniques of blastocyst division and embryo transfer. The TLO125 has a relatively large movement range and one control lever for movement in all three dimensions. A double micromanipulator unit fitted with a single left-hand tool-holder and a double right-hand tool-holder is generally used in combination with a higher magnification stereoscopic microscope. Glass micropipettes are used as tools and connected to micrometer syringes to give controlled suction for holding and manipulating the cells. Research Instruments also produces microforges, micropipette pullers and micrometer syringes.

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● Degussa has extended its range of electroplating equipment. The TG 1 series has been replaced by the TG2. This includes a new plastic unit that can accept five 2-litre vats. The new TG 2000 has a combined rectifier and temperature controller. The series TG 5 (5-litre) and TG 20 (20-litre) units have also been modified. For these bath volumes, the larger TG 2011 rectifier (10 V, 25 A) is available with current and voltage stabilizing. The 5-litre and 20-litre plastic vats are available in four versions; type A with cathode rod movement and anode/cathode system; type B with an anode/cathode system alone, type C without equipment and type D with water feed, shutoff tap and overflow. Small drums, filters, anodes and pH meters are offered as accessories to the TG range.

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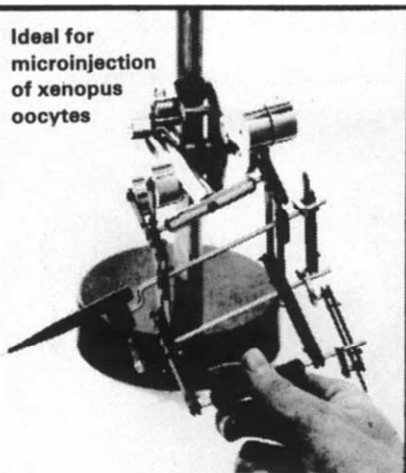
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● Wild Heerbrugg has developed a new modular photomicrographic system, the MPS 12, which complements the existing range of Wild systems. Four new cameras are available that make photomicrography and photomacrography possible at four levels of automation. The cameras are: the MPS 12 Microcamera without exposure meter; the MPS 05/12 Mikrophot, which consists of a camera with exposure meter; the MPS 15/12 Semiphotomat (semi-automatic system) and the MPS 55/51; 45/51 and 45/51S Photoautomats.

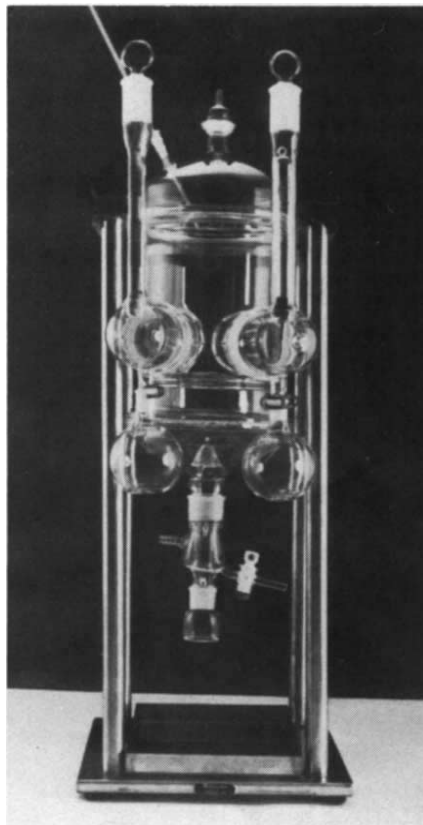
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● BioSciences Information Service (BIOSIS) has announced that a new micro-computer product, BIOSIS information transfer system (B-I-T-S) is now available. B-I-T-S will provide a means for the computerized maintenance of personal users' information. B-I-T-S subscribers will receive disks or tapes on a monthly basis. B-I-T-S is offered in several distribution options including Micro/B-I-T-S, which provides disks for personal or micro-computers, and Macro/B-I-T-S which provides tapes for main-frame or mini-computers and allows multi-site use.

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● A series of glass metabolism cages is now offered by Harvard Bioscience for use in looking at life functions in small animals. The cages give a controlled environment where the animal's urine and excretory pattern, as well as its expired air, can be studied.

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Harvard Bioscience animal cages

● Filtron-X from National Diagnostics is a new liquid scintillation solution that dissolves Millipore-type filters (cellulose acetate, cellulose nitrate and mixed ester). It renders soluble a variety of samples contained on the filter. The use of Filtron-X assures full 4π geometry counting of samples. The best results can be obtained when tritium is used as the label, since absorption of the emitted beta ray by the filter itself is completely avoided. Filtron-X will render the sample itself soluble assuring homogeneous counting. It may be used with either wet or dry filters.

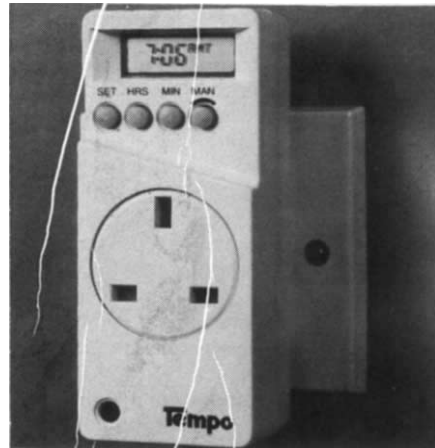
Circle No.113 on Reader Service Card.

● Two microdialysers are now available from Cambridge Biotechnology Laboratories. The microdialyser comprises three main parts — buffer circulating plate, sample chamber plate and safety lid. Two models are available: a four-chamber version, which accepts samples from 100 μ l to 1.5 ml and uses standard 62-mm diameter membranes, and a 24-chamber unit, which takes 100 μ l to 2.5 ml samples and accepts 150-mm diameter membranes. Six pre-cut ultrafilter membranes can be used to give a range of molecular weight cutoffs from 500 to 20,000.

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● A new plug-in timeswitch is now available from Tek Marketing. It has three on/off independent programmes and a fully-protected memory. The Tempo timeswitch has a microchip time circuit and is battery-powered.

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The timeswitch from Tek Marketing

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BEHAVIOUR PATTERNS OF BLOOD-SUCKING FLIES

Pergamon Press regret to announce that due to a printing error the incorrect author's initials were included on the cover and title page of this book, which was written by Dr. R.C. Muirhead-Thomson of the Royal Holloway College, University of London. The Publisher apologizes to the author and to all whom it may concern for this unfortunate mistake.

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